

Original Article

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Characterising Dutch forests, wetlands and cultivated lands on the basis of phytolith assemblages

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Abstract

Palaeoecological reconstructions in the Netherlands are commonly based on pollen and macrofossil analysis, but can be limited if the preservation of organic material is poor. Phytoliths, biogenic silica, do not have this limitation and preserve in settings where other macro- and microfossils do not. Little is known about how phytolith assemblages preserved in soils and sediments reflect the parent vegetation in north-western European systems, so it is currently difficult to contextualise past environments. Here, we characterise phytolith assemblages for soil samples recovered from three major vegetation types in the Netherlands to provide reference data for future reconstructions of past vegetation change. We collected 42 soil surface samples from forests, wetlands and agricultural fields across the Netherlands and characterised the phytolith assemblages they contained. We identified the different phytolith morphotypes and quantified the percentages and concentrations (#phytoliths/cm³ soil) in each sample. We used non-metric multidimensional scaling to assess the variation in phytolith assemblage composition within, and between, the three vegetation types. The phytolith assemblages analysed from the forests, wetlands and agricultural fields were clearly distinguishable from each other. Agricultural fields were dominated by four phytolith morphotypes of grass silica short cells (GSSCs): rondel (tabular), cross type 1 (>15 µm), rondel (elongated) and disturbance or crop phytoliths. Forests settings had significantly higher amounts of different arboreal phytoliths (large and small spheroid rugose) compared with other vegetation types. Wetlands could be identified by significantly higher amounts of Cyperaceae phytoliths (papillate) and other GSSCs (saddle and bilobates with thick castula). Phytolith assemblages could distinguish different subtypes of vegetation within forest and wetland areas, while differences between agricultural systems could not be identified. Our study demonstrates that phytoliths preserved in soils or sediments can be used to separate major vegetation types across the Netherlands. Thus, these results support the hypothesis that phytoliths can be used to infer past environmental conditions in palaeoecological reconstructions. We suggest that future work should: (1) focus on characterising which phytolith types are produced by the commonest tree, wetland, shrub and herb species in the Netherlands and (2) characterise phytolith assemblages across a wider array of vegetation types in north-western European systems to increase the capability for quantitative reconstructions using phytolith assemblages.

Introduction

Reconstructions of past environmental conditions in the Netherlands have been traditionally based upon the analysis of pollen and macro-fossil remains found in soils and sediments (e.g. van Geel et al., 1989; Janssen, 1972; Engels et al., 2016). These approaches, however, rely on the preservation of organic material which limits the geographic spread of their use (Hevly, 1981; Birks & Birks, 2000). Conversely, phytoliths are siliceous microfossils formed in vegetative structures of many different plants which preserve in a wide range of environments (Jones & Handreck, 1967; Rovner, 1971; Rovner, 1983; Shakoor et al., 2014). They are produced in high concentrations in the epidermal tissue of grasses and many herbaceous plants (Rovner, 1983), and in lower concentrations in coniferous and deciduous trees (Geis, 1973; Klein & Geis, 1978). Morphological variation of phytoliths can be used to distinguish plant types (e.g. trees, grasses, shrubs, herbs), and this variation has been used to reconstruct past vegetation changes in palaeoecological and archaeological studies (Piperno, 1985; Piperno, 2006; Piperno, 2014).

Phytoliths are sometimes paired with pollen to determine vegetation reconstructions, as both proxies show different aspects of the vegetation (e.g. Åkesson et al., 2021; Groff et al., 2022; Ramírez et al., 2019). Pollen grains are dispersed by wind, insects and larger animals and pollen assemblages captured in lake sediments typically reflect regional vegetation. Phytoliths do not

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share these dispersal mechanisms and are representative of local vegetation (Blinnikov, 2005). A combination of pollen and phytolith analysis from the same setting is possible with many lake sediment cores. Pollen, however, is especially susceptible to degradation in alkaline and hyposaline conditions (Phuphumirat et al., 2015) or in oxidative settings, such as characteristic of many soils. The silica-based phytoliths do not typically degrade in soils. Plant macrofossils, a third proxy to determine vegetation reconstruction, are also representative of local vegetation (Mauquoy et al., 2010). Plant macrofossil assemblages can provide a detailed picture of local vegetation on a species level, but have the disadvantage that they are produced in a smaller amount than pollen or phytoliths and thus larger quantities of sediment are needed for a proper analysis (Birks, 2007). Therefore, analysis of phytoliths could be beneficial either alongside macrofossils to create a more complete overview of past vegetation or as the sole proxy to determine local vegetation when macrofossil analysis is not possible.

The morphologies, or different forms, of phytoliths can vary between species, genera or families. The morphological variation of phytoliths can often be used to identify plants at the family level (Ollendorf, 1992; Piperno, 1985; Piperno, 2006; Piperno, 2014; Rovner, 1983; Shakoor et al., 2014). There are exceptions, however, and some morphotypes can be used to determine tribes or subtribes within families, such as with the Poaceae (grasses) or Cyperaceae (sedges). This makes phytolith analysis particularly complementary to pollen analysis, which cannot distinguish tribes or subtribes within the grasses or sedges. Determining Poaceae to a more specific level gives additional relevant information on past land use. For example, phytoliths make it possible to distinguish different crops within the Poaceae family, and can provide the determination of cereals to the species level using the identification of glume epidermis (Ball et al., 2016). The ability to identify cultivated grasses also makes phytolith analysis exceptionally useful for archaeological research.

Pollen or phytolith assemblages from surface soils or sediments reflect modern vegetation and are commonly used as a foundation to quantify past environmental change (e.g. Birks, 2019; Delhon et al., 2003; Odgaard, 1999; Watling et al., 2016; Zhang et al., 2018). Phytolith assemblages from soil surface samples and phytolith morphotypes from herbarium samples have been characterised for different parts of the world, such as eastern Europe (Alexandra, 1997), the Mediterranean region (Bremond, et al., 2004), North America (Blinnikov, 2005; Fredlund & Tieszen, 1994), Africa (Runge, 1999) and South America (e.g. Dickau et al., 2013; Huisman et al., 2018; Witteveen et al., 2022). In North-Western Europe, analysis of surface sample phytolith assemblages is limited (Powers et al., 1989), particularly in the Netherlands (McMichael et al., 2019). Filling the knowledge gap on phytolith assemblages from surface samples, and how they relate to the modern vegetation and environment in North-Western Europe and specifically in the Netherlands, would allow more detailed and quantifiable reconstructions of past ecological change in these areas. By creating a link between modern vegetation compositions and their corresponding phytolith assemblages, characterising and quantifying past environmental changes using phytoliths become possible. Here, we assess the variability of phytolith assemblages within and between vegetation types (forest, agricultural and wetland), compare the abundances and concentrations of phytolith types between vegetation types and highlight the value of phytolith analysis in studies of past environmental change in the Netherlands.

Methods

Experimental design

Currently, more than 50% of the Netherlands have been classified as agricultural land, 8% as forested and ca. 4% as wetland (CBS et al., 2016). The other 34% has been classified as water (19%), urban areas (13%) and recreational areas (2%) (CBS et al., 2016) (Fig. 1). We collected 42 soil surface samples from 21 locations (2 samples per locations) throughout the Netherlands in December 2017 and January 2018 (Table 1 and Fig. 1). Our sampling included forests, agricultural fields and wetlands, each consisting of 14 samples and 7 locations (Table 1). The location of the sites was chosen to evenly cover the Netherlands for each vegetation type. Additionally, forest samples were chosen based on their “plantgemeenschap” (Schaminée et al. 2010), agricultural fields on the type of crop that was grown and wetlands on the type of wetland (Scott & Jones, 1995). All samples were collected from the top layer of the soil (0–1 cm) directly below the litter layer.

Soils in the Netherlands commonly consist of peat, sand, clay and loam (Alterra, 2006), and our sampling design consisted of nine sites with peat or peaty sediment, nine sites with sandy soils, two with clay soils and one site with loam (Table 1). The average annual precipitation (average of 1991–2020) in the Netherlands is 862 mm, and at our sampled sites ranged from 800 to 925 mm per year (CBS et al., 2020a). The average annual temperature in the Netherlands is 10.5°C, though at our sampled sites ranged from 10.1 to 11.1°C (CBS et al., 2020c).

Site description

Two forests included in this study are characterised as deciduous forest on rich soil; Amerongse Bos and Norgerholt. The Amerongse Bos, located in the southern part of the province Utrecht, consists of a combination of *Quercus*, *Picea* and *Betula*, all situated on a sandy soil. Norgerholt is a unique forest with mainly *Ilex* shrubs alongside *Quercus* trees on an organic rich soil. Contrary to the Amerongse Bos and Norgerholt are Meijendel and Sprielder Bos deciduous forests on poor soil. Meijendel, located near the coast in Zuid-Holland, mostly consists of both *Pinus* and *Quercus* on a peaty soil while Sprielder Bos, in the north-western part of Gelderland, has a combination of *Quercus* and *Fagus* on a sandy soil. Spanderswoud, in the south-eastern part of Noord-Holland, is also a deciduous forest but has an intermediate soil type. This forest, with a peaty soil, has a combination of *Quercus*, *Fagus* and *Betula* trees. Dwingelderveld, situated in the province Drenthe, is mainly dominated by *Pinus* trees, thus making this the only coniferous forest in this study. This forest has very little undergrowth with a well-developed moss layer and is situated on a sandy soil. The only wet forest, Alde Feanen, is located in the North of the Netherlands (Friesland). Alde Feanen has a rather wet soil and is, aside from a forest, also partly a wetland. The forest part of Alde Feanen is dominated by *Betula* trees and has a peat soil.

On three of the agricultural fields maize (*Zea mays*) is cultivated; Winterswijk, Plasmolen and Wouwse Plantage. Winterswijk is located near the German border in Gelderland, Plasmolen also near the German border but more South in Noord-Brabant and Wouwse Plantage near the coast in the south of Zuid-Holland. All three locations have a sandy soil and lay next to patches of forest. Groenekan, in the centre of the province Utrecht, has a sandy soil as well but cereal (*Cerealia*) is cultivated here. East to Groenekan, near the Veluwe in Gelderland, lays



Fig. 1. Locations used in phytolith composition comparisons in the Netherlands. The colours of the circles (study sites) indicate the main vegetation type, and the background map shows the variation in land use (CBS et al., 2020b).

Putten. Here, sugar beets (*Beta vulgaris*) and potatoes (*Solanum tuberosum*) are cultivated on an organic-rich soil. Sugar beets are cultivated in Norg as well. This location is situated in the North of the Netherlands in Drenthe. The last agricultural field in this study is situated near the town Enschede in East of Overijssel. This field is a grassland on a loamy soil.

Two of the wetlands in this study can be classified as open peat bog: Wieden and Weerribben, which are located next to each other in the Northern province Friesland. These two wetlands are dominated by Poaceae and Juncaceae. A similar wetland also in Friesland is Alde Feanen. This wetland is a combination of an open peat bog and a freshwater lake and is situated next to a wet forest. Here, a combination of Poaceae, Juncaceae and some small patches of trees and shrubs can be found. Markiezaat, in the western part of the Netherlands, is a nature reserve around a freshwater lake. The Markiezaatsmeer used to be part of the Oosterschelde but has been closed off in 1984, converting the lake to a freshwater marsh. The area around the lake is dominated by Poaceae and has some large herbivores present that graze on the plants in the area. These grazers are also present in the Oostvaardersplassen, a nature reserve in the

province Flevoland. This area is relatively young since the province only exists since the 1960s. The wetland can be classified as a swamp forest. A different class of forested wetland is the tidal forest which we see in the Biesbosch in the west of Noord-Brabant. This wetland is located at the end of an estuary and thus is a freshwater wetland. The only saltmarsh is Saeftinghe, which is an area that used to be populated but has been submerged during the 14th and 16th century.

Laboratory analysis

All soil surface samples ($n = 42$) were processed for phytoliths at the Palaeoecology Laboratory at the University of Amsterdam (UvA), using a subsample containing 1 cm³ of soil. At the onset of laboratory processing, 56,000 microspheres (Microparticles GmbH, Lot: SiO₂-R-L3519-3, ϕ 15.29 μ m, SD 0.49 μ m) were added to each sample to calculate phytolith concentrations (Huisman et al., 2019; Witteveen et al., 2022). A series of chemical treatments, with 33% hydrogen peroxide (H₂O₂), 10% hydrochloric acid (HCl) and potassium manganate (KMnO₄), were performed on each

Table 1. Locations of soil surface samples collected in the Netherlands and analysed for phytoliths. Samples were collected in 21 locations (n = 2 for each location) throughout the Netherlands from three different vegetation types. Coordinates (latitude, longitude), soil type, average annual precipitation (Precip.) and average annual temperature (Temp.) are given per location (CBS et al., 2020a; CBS et al., 2020b, CBS et al., 2020c). Forests are categorised by “plantgemeenschap” (Schaminée et al., 2010) (per location) and most commonly occurring tree species (per sample), agricultural fields by the type of crop and wetlands by wetland type (Scott & Jones, 1995).

Location	Latitude	Longitude	Vegetation type	Soil type	Precip. (mm)	Temp. (°C)	Vegetation category
Spanderswoud	52.252737	5.141091	Forest	Peaty	925	10.6	Deciduous forest, nutrient rich; 1. <i>Quercus</i> 2. <i>Fagus</i> & <i>Betula</i>
Norgerholt	53.057718	6.453474	Forest	Peaty	850	10.1	Deciduous forest, nutrient rich; 1. <i>Quercus</i> 2. <i>Quercus</i> & <i>Ilex</i>
Amerongse Bos	52.008924	5.479102	Forest	Sand	875	10.6	Deciduous forest, medium nutrient; 1. <i>Quercus</i> 2. <i>Picea</i> & <i>Betula</i>
Mijndel	52.126805	4.340589	Forest	Peaty	925	10.8	Deciduous forest, nutrient poor; 1. <i>Pinus</i> & <i>Quercus</i> 2. <i>Pinus</i>
Spielder Bos	52.263171	5.669217	Forest	Sand	875	10.6	Deciduous forest, nutrient poor; 1. <i>Quercus</i> & <i>Fagus</i> 2. <i>Fagus</i>
Alde Feanen	53.138513	5.946824	Forest	Sand	825	10.3	Wetland-like forest; 1. <i>Betula</i> 2. <i>Betula</i>
Dwingelderveld	52.831877	6.449874	Forest	Sand	850	10.3	Coniferous forest; 1. <i>Pinus</i> 2. <i>Pinus</i>
Oostvaardersplassen	52.453835	5.416287	Wetland	Clay	850	10.6	Swamp forest
Biesbosch	51.77874	4.76838	Wetland	Clay	850	10.8	Tidal forest
Wieden	52.685533	6.007365	Wetland	Peaty	850	10.6	Open peat bog/ fen
Weerribben	52.783703	5.966939	Wetland	Peaty	850	10.6	Open peat bog/ fen
Alde Feanen	53.118777	5.953514	Wetland	Peaty	825	10.3	Open peat bog/ fen; Freshwater lake
Markiezaat	51.46822	4.28553	Wetland	Peaty	825	10.9	Freshwater lake
Saefinghe	51.33502	4.18496	Wetland	Peaty	825	11.1	Saltmarsh
Winterswijk	52.01461	6.75399	Agriculture	Sand	825	10.6	Maize
Wouwe Plantage	51.47602	4.39351	Agriculture	Sand	875	10.9	Maize
Plasmolen	51.75149	5.91623	Agriculture	Sand	800	10.8	Maize/ cereal
Groenekan	52.12953	5.17421	Agriculture	Sand	900	10.6	Cereal
Enschede	52.26481	6.91306	Agriculture	Loam	825	10.5	Grassland
Norg	53.08248	6.49485	Agriculture	Peaty	850	10.1	Sugar beet
Putten	52.23327	5.68016	Agriculture	Sand	875	10.6	Sugar beet/ potato

sample. Bromoform (CHBr₃, 2.3 SG) was then added to the samples, and the mixture was centrifuged at 1500 rpm for 10 minutes (McMichael et al, 2021). Phytoliths and microspheres were separated from heavier materials and thus got collected in the top of the mixture. This top part was poured into a new tube with 100% ethanol and centrifuged twice at 4500 rpm for 1.5 minutes. Afterwards, phytoliths were mounted on microscope slides in Naphrax permanent mountant. The extracted phytoliths were then mounted on microscope slides in Naphrax and stored at the University of Amsterdam Palaeoecology Laboratory as reference material.

Data analysis

All phytolith morphotypes were identified using literature (Blinnikov, 2005; Pearsall, 2015; Piperno, 2006), and the phytolith catalogue (phytolith reference material) of the University of Amsterdam, and were counted using a light microscope (Carl Zeiss axioscope microscope) at 400x magnification. The microspheres were counted simultaneously to determine the concentration of phytoliths. At least 350 phytoliths or 1000 microspheres were counted per sample, of which at least 200 of the 350 phytoliths

needed to be grass silica short-cell phytoliths (GSSC) (Aleman et al., 2014). Percentages and concentrations per phytolith morphotype were calculated. The concentration of the phytoliths was calculated using the count of the added microspheres and eqn 1.

$$\text{Concentration} = \frac{\text{total added microspheres} \cdot \text{counted\# phytoliths}}{\text{counted\# microspheres}} \quad (1)$$

We also calculated the total concentration of phytoliths (all morphotypes combined), and concentrations and percentages for phytolith groups, including: (1) the sum of arboreal phytoliths (spheroid rugose (large), spheroid rugose (small), spheroid ornate and elongate entire), (2) the sum of grass phytoliths (rondel (wide), rondel (elongated), rondel (tabular), tent-shaped body, trapezoid, polylobate (symmetrical), polylobate (asymmetrical), bilobates (thin castula), bilobate (thick castula), saddle and cross) and (3) the sum of herbaceous phytoliths (papillate, Asteraceae, spheroid

psilate, elongate dendate and elongate dendritic) (Blinnikov, 2005; Pearsall, 2015; Piperno, 2006).

Non-metric Multidimensional Scaling (NMDS) was performed on the phytolith percentages and concentrations to assess the similarities and dissimilarities within and between sites and vegetation types. Morphotypes were excluded from the ordination analysis if they occurred in less than two samples or less than 5% abundance in total. We also performed one-way analyses of variance (ANOVAs) to identify differences in individual phytolith morphotypes and phytolith morphotype groups between vegetation types (forest, agricultural field, wetland). A Tukey HSD post-hoc test was used when the one-way ANOVA showed significant differences between groups. When needed, a log-transformation or a sqrt-transformation was used to meet the assumptions of the ANOVA. When the assumptions for an ANOVA were not met after transformations, a Kruskal–Wallis test was performed instead with a Dunn test as corresponding post-hoc test. All phytolith data were analysed using R version 3.6.3 (R Core Team, 2013) and R-studio version 1.2.5033 (R Team, 2015).

Results

The total percentages and concentrations of arboreal (forest), grass and herbaceous phytoliths were significantly different between forest, agricultural and wetland vegetations (Table A1; Figs. 2 and 3). Forest vegetations contained significantly lower total grass phytolith percentages (Table A2) than agricultural fields or wetlands, and significantly higher arboreal phytolith percentages (Tables A1 and A2; Fig. 2). Forest samples had significantly lower total phytolith concentrations than wetlands and agricultural fields, and lower total grass phytolith and herbaceous phytolith concentrations (Tables A1 and A2; Fig. 3). Wetland vegetations contained significantly higher total herbaceous phytolith percentages than forests and agricultural fields (Tables A1 and A2; Fig. 2). Wetlands had the highest total herbaceous phytolith concentration (Tables A1 and A2; Fig. 3). There were no significant differences between the total or total grass phytolith concentrations between wetlands and agricultural fields (Table A1).

Forested vegetations had significantly higher percentages of all types of arboreal phytoliths, and significantly lower percentages of tent-shaped bodies, a morphotype representing grass phytoliths (Tables A1 and A2; Fig. 2), compared with wetlands and agricultural fields. Concentrations of grass phytolith morphotypes were consistently low within forest vegetations (Fig. 3). The concentrations of five types of grass phytoliths were significantly lower in forests compared with agricultural fields and wetlands: tent-shaped bodies, tabular rondels, elongated rondels, trapezoids and symmetrical polylobates (Tables A1 and A2; Fig. 3). Concentrations of elongate dendate phytoliths, produced by herbaceous taxa, were also significantly lower in forests compared with other vegetation types.

The percentages of tabular rondels, crosses (small and large type 1) and elongated rondels, which represent grasses, were significantly higher in agricultural fields compared with wetlands or forest vegetations (Tables A1 and A2; Fig. 2). The percentages of spheroid ornate phytoliths, which are arboreal but are usually linked to disturbed areas, and elongate dendate phytoliths, which represent herbaceous taxa, were also significantly higher in agricultural fields compared with wetland or forest vegetations (Tables A1 and A2; Fig. 2). The agricultural fields contained significantly lower percentages of spheroid psilate and elongated dendate phytoliths than other vegetation types. Four phytolith morphotypes occur

in significantly higher concentrations in agricultural fields compared with wetlands or forests: elongate dendritic, bilobates with thin castula, crosses and spheroid ornates (Tables A1 and A2; Fig. 3). Asteraceae phytoliths occurred in smaller amounts in agricultural fields than in wetland and forest vegetations (Figs. 2 and 3).

Wetlands contained significantly higher percentages and concentrations of bilobate (thick castula), saddles and papillates (Cyperaceae) phytoliths compared with agricultural field and forest vegetations (Tables A1 and A2; Figs. 2 and 3). The concentrations of elongate entire, which represent herbaceous taxa, and Asteraceae, which is a shrub, are significantly higher in the wetland vegetation samples compared with other vegetation types (Fig. 3).

The NMDS analysis showed that the entire phytolith assemblages were distinguishable between forest, agricultural and wetland vegetation types using both the percentages (Fig. 4A–E) and concentrations (Fig. A1A–E) data. This separation was clear on the first ordination axis (NMDS1). Negative NMDS1 scores corresponded with forested settings, and positive scores corresponded with wetland settings (Fig. 4A). Agricultural fields were located in the middle of NMDS1, as they contained taxa found in both forest and wetland vegetations and were distinguishable by their high abundances of grass phytolith morphotypes (Fig. 4A). The second ordination axis (NMDS2) separated the forest and wetland samples, which contained negative scores, from the agricultural fields, which contained positive scores (Fig. 4A). The separation on NMDS2 was primarily driven by differences in the percentages of crosses (type 1 large) and Asteraceae phytolith morphotypes (Fig. 4A).

The agricultural fields showed less within-vegetation variation in phytolith assemblages than the forest and wetland samples (Fig. 4B–D). The ordination scores from the wetland samples were driven by a gradient of forested to open wetlands (Fig. 4C) and the scores of the forested samples were loosely based on the richness of the soils (Fig. 4D). No clear separation could be seen within forests between most commonly occurring tree species (Fig. 4E). Results of the ordination using the phytolith concentration data (Appendix A, Fig. A1A–E) were similar to the results using the percentage data (Fig. 4A–E).

Discussion

The need for more phytolith reference material across Europe due to interregional variability and environmental heterogeneity has been previously noted (Zurro et al., 2016). Most major vegetation types in the Netherlands were covered in our survey except in the south of the Netherlands (Limburg), which has a different type of soil (loess) and vegetation compared with the northern part of the country (Alterra, 2006; Bazelmans et al., 2011). Our comparisons of specific phytolith morphotypes and the phytolith assemblage data clearly showed that the forest, agricultural and wetland vegetations within our sampling were clearly distinguishable using the percentage or concentration data, and that there were patterns of variability both within and between sites (Figs. 2–4).

Even though many tree families found in the Netherlands do not produce many phytoliths (Piperno, 2006), our results showed that these taxa still produce enough phytoliths to distinguish forested from non-forested settings (Figs. 2–4). Forest types as defined by Schaminée et al. (2010) (Table 1; Figs. 4D and A1C) showed clustering in the NMDS, but clustering based on the most commonly occurring tree species was not evident (Fig. 4E and A1D). These results suggest that gradients in soil type and nutrient

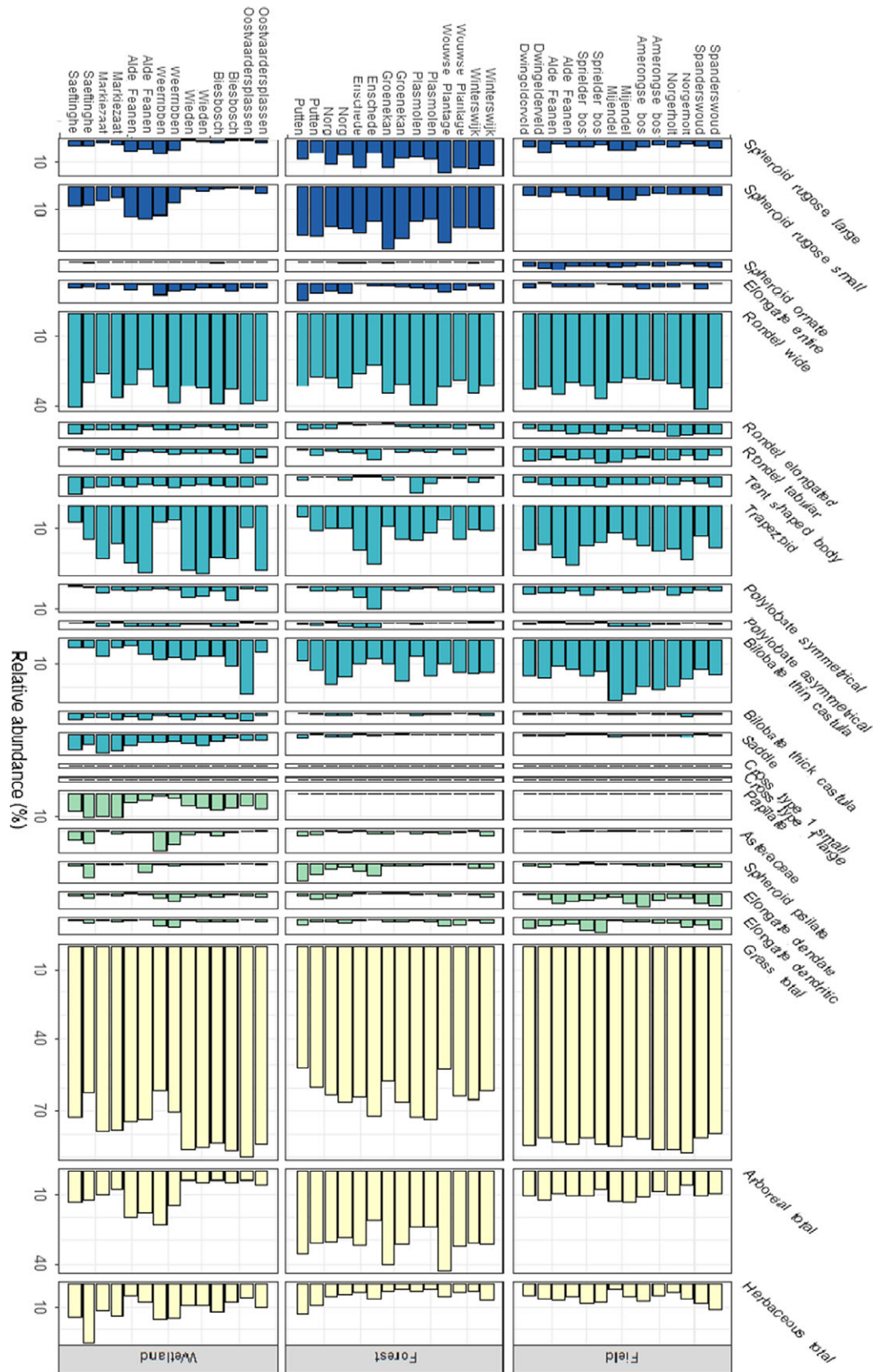


Fig. 2. Relative abundances of phytolith morphotypes (%) recorded within forests, agricultural fields, and wetlands across the Netherlands. Dark blue phytolith morphotypes represent arboreal taxa, light blue morphotypes represent grass taxa, and green morphotypes represent herbaceous taxa. Yellow columns show the sums of arboreal, grass, and herbaceous phytolith morphotypes.

levels (Table 1) likely drive differences in the phytolith assemblages (Fig. 4D and A1C), and that the less common trees or undergrowth species are the major phytolith producers in forested vegetations.

In other parts of the world, forest types have been shown to be distinguishable from other vegetation types and from each other. For instance, studies conducted in a temperate region in North-eastern

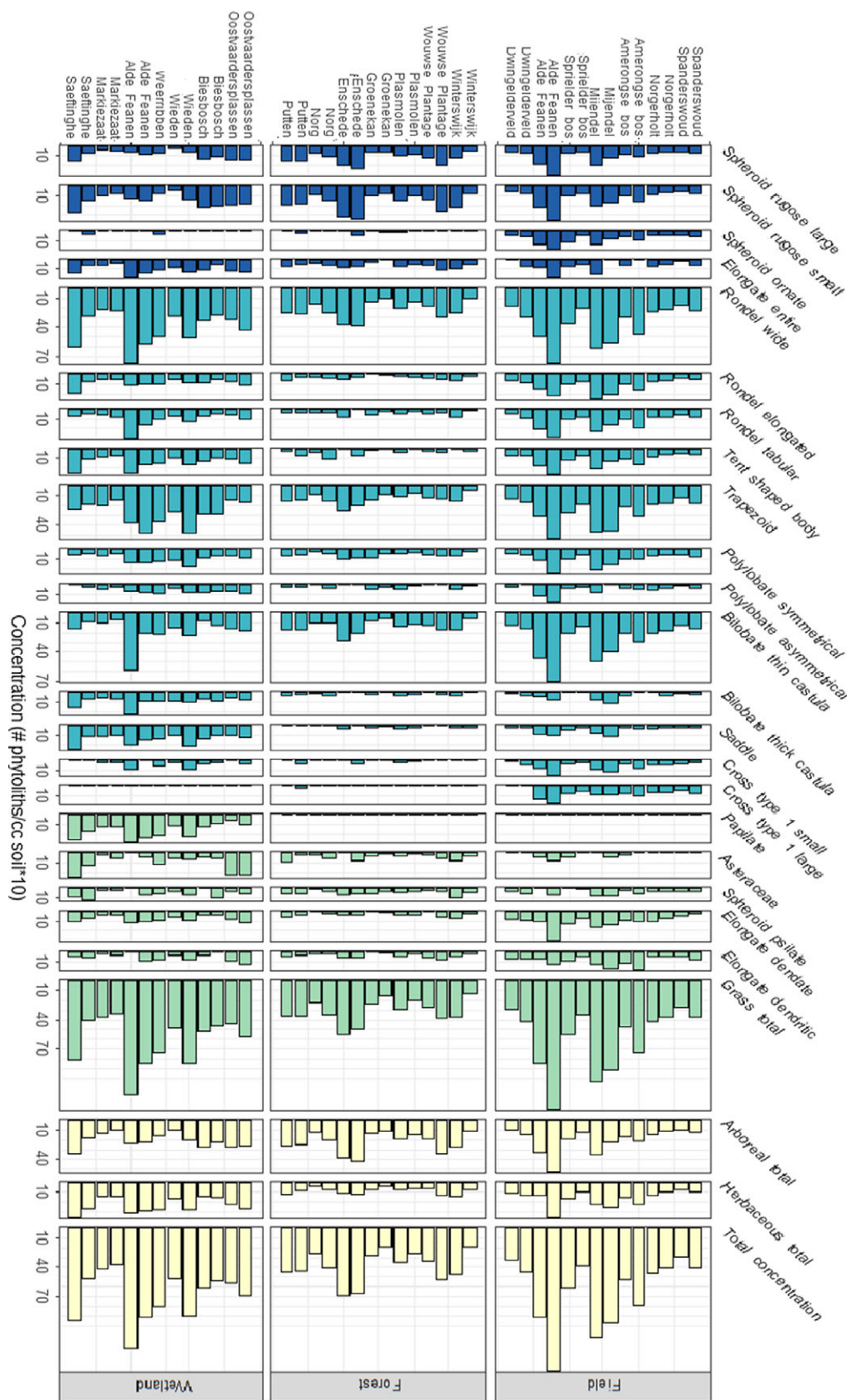


Fig. 3. Phytolith concentrations (#phytoliths/ cm³ soil, square root transformed) recorded within forests, agricultural fields, and wetlands across the Netherlands. Dark blue phytolith morphotypes represent arboreal taxa, light blue morphotypes represent grass taxa, and green morphotypes represent herbaceous taxa. Yellow columns show the sums of arboreal, grass, and herbaceous phytolith morphotypes.

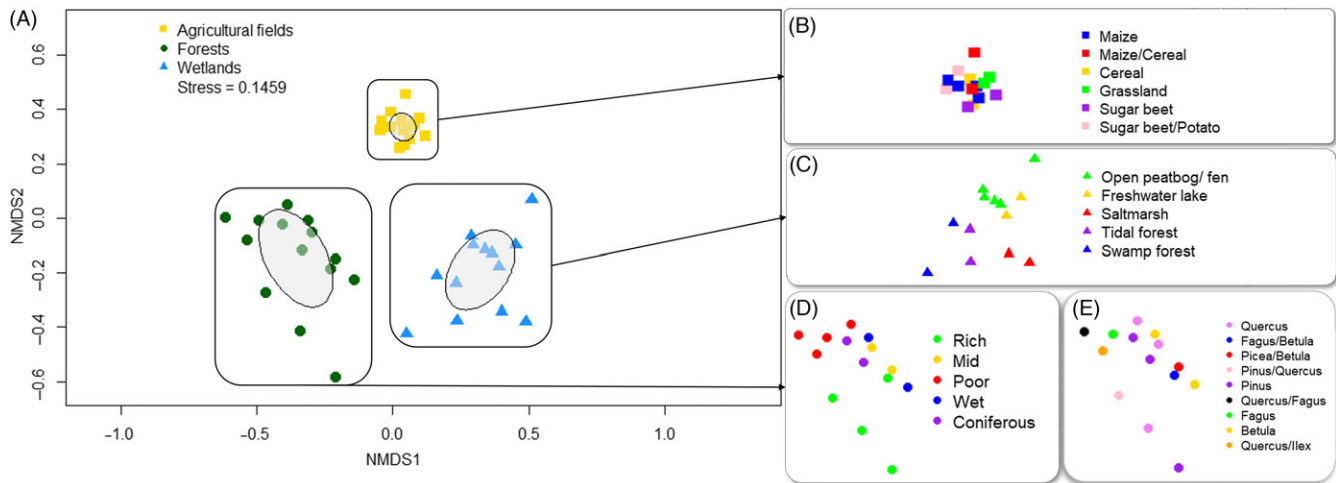


Fig. 4. NMDS of phytolith samples. The NMDS was carried out using the percentages (%) in which each morphotype is present (stress = 0.1459). A: overall results of the NMDS, blue dots represent the samples taken from wetlands, the green dots the samples taken from forests and the yellow dots the samples taken from agricultural fields. The circles show the centre of the different clusters. B–E: within variation of the different vegetation types: B – agricultural fields, C – wetlands, D – forests (forest type based on Schaminée et al. (2010)), E – forests (most commonly occurring tree species).

China have found that woody communities could be differentiated from herbaceous and grass-dominated communities using phytoliths (Gao et al., 2018; Gao et al., 2019). Additionally, they found that different forest types, such as coniferous forests and deciduous forests, could be differentiated from each other. Another study found that the assemblages of phytoliths are strongly correlated with soil type (Hyland et al., 2013), similarly to our results within the different forest types.

Like forests, there is also a wide variety of wetland vegetations. The Convention on Wetlands of International Importance especially as Waterfowl Habitat (Ramsar Convention Bureau, 1971) has defined wetlands as ‘areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water the depth of which at low tide does not exceed six meters’. Due to this broad definition, at least 22 different types of wetlands occur in the world (Scott & Jones, 1995), with at least five different types occurring in the Netherlands. The phytolith assemblages from the wetland systems of the Netherlands were clearly distinguishable from other vegetation types because of the high abundances of papillate morphotypes (Figs. 4A and A2), which are produced by Cyperaceae and Juncaceae species (Ollendorf, 1992; Piperno, 2006). As with the forest phytolith assemblages, the wetland phytolith assemblages also varied along environmental gradients within the vegetation types. The NMDS indicated a gradient from freshwater to saltwater wetlands and from forested to open wetlands (Fig. 4C). For instance, saltwater wetlands had higher percentages of the phytolith morphotypes Asteraceae than freshwater wetlands, a family that contains species generally associated with salt marshes (e.g. *Tripolium pannonicum*, *Artemisia maritima*) and species generally associated with open vegetation types. Since Dutch agricultural fields usually weedless, this might explain why the Asteraceae-phytoliths are found less in the agricultural fields. Forested wetlands contained more arboreal morphotypes than open wetlands and less papillate morphotypes, generally linked to Cyperaceae and Juncaceae (Figs. 2, 3, and A2).

We expect that additional analysis of soil surface samples would show that the various types of forested and wetland vegetations within north-western Europe have distinguishable phytolith

assemblages driven by soil nutrient and hydrological gradients, similar to the results of Hyland et al. (2013). Phytolith assemblages from soil surface samples in other regions are also known to reflect these environmental gradients (e.g. North America, South America, Eastern Europe and Asia) (Alexandra, 1997; Blinnikov, 2005; Bremond et al., 2004; Dickau et al., 2013; Gao et al. 2018; Gao et al., 2019).

The agricultural fields showed less variability in phytolith assemblages between sites than the forested or wetland vegetations (Fig. 4B–E). The NMDS showed no separation or patterning of the agricultural sites based on the type of crop grown on the fields. The agricultural fields could be determined by high amounts of grass silica short cell phytoliths (GSSCs) and phytoliths linked to crops and disturbance (Figs. 2 and 3), which has also been found in phytolith analysis from other agricultural fields (Ball et al., 2016). While we did find some maize phytoliths (large type 1 crosses) in the agricultural fields, they were not very abundant. Interestingly, maize phytoliths were not limited to field that grew maize at the moment of sampling but also in some other fields, this thus might indicate the act of crop switching on several of the sampled fields. In the agricultural field sites, we are also unsure of how much plant material is harvested and removed, and how much of the soil surface is overturned, which may affect the phytolith assemblages and low abundances of maize phytoliths. Large differences in the total concentrations of the agricultural fields were found, implying that some fields may have more material removed than others (e.g. mechanised versus non-mechanised removal of plant material).

Our results demonstrate that phytolith assemblages in the Netherlands reflect environmental variability and heterogeneity and would thus be a valuable tool in reconstructing environmental change through time. While humans have impacted modern ecosystems drastically, our results are still applicable to past vegetation reconstructions since we characterise major vegetation types by large differences in phytolith composition and not by specific amounts (percentages of concentrations) of phytoliths per vegetation type. This method thus results in a robust characterisation of phytolith compositions in forests, wetlands and agricultural fields in the Netherlands, and in similar systems in north-western Europe.

Pollen is the most common microfossil used in qualitative and quantitative palaeoecological reconstructions of vegetation change (e.g. Birks et al., 2016a; Birks et al., 2016b; Weng et al., 2007). Phytoliths, however, can preserve in palaeoecological archives where pollen or macrofossils or macrofossils degrade via oxidation (Piperno, 2006). Pollen and phytoliths can be analysed together to provide several complementary aspects of past vegetation change as has been demonstrated in other parts of the world (e.g. Åkesson et al., 2021, Gao et al., 2018; Gao et al., 2019). Pollen provides signals of regional vegetation whereas phytoliths provide signals of local vegetation. Pollen can be used to identify the major tree species in a landscape, but cannot distinguish various types of grasses (Poaceae) or sedges (Cyperaceae) to a degree that can be achieved with phytoliths. While phytoliths give the same local vegetation signal as macrofossils, they can be especially useful when macrofossil analysis is not possible. For example, when too little material is available for this type of research or when no macro remains are preserved in the soils or sediments. The optimal scenario of capturing local and regional vegetation changes from the same site would be through a combination of phytolith, pollen and macrofossil analyses.

Conclusions

Our study demonstrates the practicality of the use of phytoliths in soils and sediments for palaeoecological research in the Netherlands. Distinct phytolith assemblages have been shown to distinguish between major vegetation types: forests (characterised by high amounts of arboreal phytoliths and lower amounts of several grass phytoliths), wetlands (characterised by higher amounts of Cyperaceae, Asteraceae and some grass phytoliths) and agricultural fields (characterised by lower amounts of arboreal phytoliths and higher amounts of grass and crop phytoliths). In addition, differences between wetland types and forest types were found. Phytoliths can be especially useful to reconstruct local vegetation in combination with macrofossils, or when macrofossil analysis is not possible. We suggest that future palaeoecological analyses should use all three proxies when possible to detect and quantify local and regional vegetation and past agricultural practices. The multiproxy approach can be used to achieve a more comprehensive assessment of local and regional vegetation and environmental change.

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References

- Åkesson, C.M., McMichael, C.N.H., Raczka, M.F., Huisman, S.N., Palmeira, M., Vogel, J.C., Neill, D., Veizaj, J., Bush, M.B. & de Lafontaine, G., 2021. Long-term ecological legacies in western Amazonia. *Journal of Ecology* **109**(1): 432–446.
- Aleman, J.C., Canal-Subitani, S., Favier, C. & Bremond, L., 2014. Influence of the local environment on lacustrine sedimentary phytolith records. *Palaeogeography, Palaeoclimatology, Palaeoecology* **414**: 273–283.
- Alexandra, A., 1997. Content and distribution of phytoliths in the main types of soils in eastern Europe. Paper presented at the First European Meeting on Phytolith Research, 4: 15.
- Alterra, 2006. Grondsoortenkaart. WUR. <https://www.wur.nl/nl/show/Grondsoortenkaart.htm>
- Ball, T., Chandler-Ezell, K., Dickau, R., Duncan, N., Hart, T.C., Iriarte, J., Lentfer, C., Logan, A., Lu, H., Madella, M., Pearsall, D.M., Piperno, D.R., Rosen, A.M., Vrydaghs, L., Weisskopf, A. & Zhang, J., 2016. Phytoliths as a tool for investigations of agricultural origins and dispersals around the world. *Journal of Archaeological Science* **68**(5): 32–45.
- Bazelmans, J., Weerts, H. & van der Meulen, M., 2011. Atlas van Nederland in het holoceen: Landschap en bewoning vanaf de laatste ijstijd tot nu.
- Birks, H.H. & Birks, H.J.B., 2000. Future uses of pollen analysis must include plant macrofossils. *Journal of Biogeography* **27**(1): 31–35.
- Birks, H.H., 2007. Plant macrofossil introduction. *Encyclopedia of Quaternary Science* **3**: 2266–2288.
- Birks, H.J.B., 2019. Contributions of Quaternary botany to modern ecology and biogeography. *Plant Ecology & Diversity* **12**(3–4): 189–385.
- Birks, H.J.B., Felde, V.A., Bjune, A.E., Grytnes, J.A., Seppä, H. & Giesecke, T., 2016b. Does pollen-assemblage richness reflect floristic richness? A review of recent developments and future challenges. *Review of Palaeobotany and Palynology* **228**: 1–25.
- Birks, H.J.B., Felde, V.A. & Seddon, A.W., 2016a. Biodiversity trends within the Holocene. *The Holocene* **26**(6): 994–1001.
- Blinnikov, M.S., 2005. Phytoliths in plants and soils of the interior pacific northwest. *USA Review of Palaeobotany and Palynology* **135**: 71–98. doi: 10.1016/j.revpalbo.2005.02.006.
- Bremond, L., Alexandre, A., Véla, E. & Guiot, J., 2004. Advantages and disadvantages of phytolith analysis for the reconstruction of Mediterranean vegetation: an assessment based on modern phytolith, pollen and botanical data (Luberon, France). *Review of Palaeobotany and Palynology* **129**(4): 213–228.
- CBS, PBL, RIVM & WUR, 2016. Land- en tuinbouw: Ruimtelijke spreiding, grondgebruik en aantal bedrijven, 1980–2015. Available at <http://www.clo.nl/indicatoren/nl2119-agrarisch-grondgebruik>
- CBS, PBL, RIVM & WUR, 2020a. Jaarlijkse hoeveelheid neerslag in Nederland, 1910–2019 (indicator 0508, versie 08, 24 April 2020). Available at www.clo.nl
- CBS, PBL, RIVM & WUR, 2020b. Kaart bodemgebruik van Nederland, 2015 (indicator 0061, versie 11, 8 Januari 2020). Available at www.clo.nl
- CBS, PBL, RIVM & WUR, 2020c. Temperatuur in Nederland en mondiaal, 1907–2019. Available at <https://www.clo.nl/indicatoren/nl0226-temperatuur-mondiaal-en-in-nederland>
- Delhon, C., Alexandre, A., Berger, J.F., Thiébault, S., Brochier, J.L. & Meunier, J.D., 2003. Phytolith assemblages as a promising tool for reconstructing Mediterranean Holocene vegetation. *Quaternary Research* **59**(1): 48–60.
- Dickau, R., Whitney, B.S., Iriarte, J., Mayle, F.E., Soto, J.D., Metcalfe, P., Street-Perrott, F.A., Loader, N.J., Ficken, K.J. & Killeen, T.J., 2013. Differentiation of neotropical ecosystems by modern soil phytolith assemblages and its implications for palaeoenvironmental and archaeological reconstructions. *Review of Palaeobotany and Palynology* **193**: 15–37.
- Engels, S., Bakker, M.A.J., Bohncke, S.J.P., Certi, C., Hoek, W.Z., Jansen, B. & Wolma, M., 2016. Centennial-scale lake-level lowstand at Lake Uddelermeer (The Netherlands) indicates changes in moisture source region prior to the 2.8-kyr event. *The Holocene* **26**(7): 1075–1091.
- Fredlund, G.G. & Tieszen, L.T., 1994. Modern phytolith assemblages from the north American great plains. *Journal of Biogeography* **21**: 321–335.
- Gao, G., Jie, D., Li, D., Li, N., Liu, L., Liu, H., Leng, C., Wang, J., Liu, B. & Li, P., 2019. Assessing modern arboreal phytolith sensitivity to vegetation variations in temperate forest regions. *Boreas* **48**(3): 731–745.
- Gao, G., Jie, D., Wang, Y., Liu, L., Liu, H., Li, D., Li, N., Shi, J. & Leng, C., 2018. Do soil phytoliths accurately represent plant communities in a temperate region? A case study of northeast China. *Vegetation History and Archaeobotany* **27**(6): 753–765.
- Geis, J.W., 1973. Biogenic silica in selected species of deciduous angiosperms. *Soil Science* **116**(2): 113–130.
- Groff, D.V., Greenawald, K.E. & Gill, J.L., 2022. Grass pollen and phytoliths of the Falkland islands. *Review of Palaeobotany and Palynology* **299**: 104603.
- Hevly, R.H., 1981. Pollen production, transport and preservation: potentials and limitations in archaeological palynology. *Journal of Ethnobiology* **1**(1): 39–54.
- Huisman, S.N., Bush, M.B. & McMichael, C.N.H., 2019. Four centuries of vegetation change in the mid-elevation Andean forests of Ecuador. *Vegetation History and Archaeobotany* **28**(6): 679–689.
- Huisman, S.N., Raczka, M.F. & McMichael, C.N.H., 2018. Palm phytoliths of mid-elevation Andean forests. *Frontiers in Ecology and Evolution* **6**: 193.

- Hyland, E., Smith, S.Y. & Sheldon, N.D.**, 2013. Representational bias in phytoliths from modern soils of central north America: implications for paleovegetation reconstructions. *Palaeogeography, Palaeoclimatology, Palaeoecology* **374**: 338–348.
- Janssen, C.R.**, 1972. The palaeoecology of plant communities in the Dommel Valley, North Brabant, Netherlands. *The Journal of Ecology* **60**: 411–437.
- Jones, L.H.P. & Handreck, K.A.**, 1967. Silica in soils, plants, and animals. *Advances in Agronomy* **19**(2): 107–149.
- Klein, R.L. & Geis, J.W.**, 1978. Biogenic silica in the pinaceae. *Soil Science* **126**(3): 145–156.
- Mauquoy, D., Hughes, P.D.M. & Van Geel, B.**, 2010. A protocol for plant macrofossil analysis of peat deposits. *Mires and Peat* **7**(6): 1–5.
- McMichael, C.N.H., de Wolf, I.K. & Land, K.**, 2019. Phytolith analysis of soil archives: Phytoliths in soils of the Netherlands. *In: Reading the Soil Archives: Unraveling the Geocological Code of Palaeosols and Sediment Cores*. Elsevier (Amsterdam): 251.
- McMichael, C.N.H., Witteveen, N.H., Scholz, S., Zwier, M., Prins, M.A., Lougheed, B.C., Mothes, P. & Gosling, W.D.**, 2021. 30,000 years of landscape and vegetation dynamics in a mid-elevation Andean valley. *Quaternary Science Reviews* **258**: 106866.
- Odgaard, B.V.**, 1999. Fossil pollen as a record of past biodiversity. *Journal of Biogeography* **26**(1): 7–17.
- Ollendorf, A.L.**, 1992. Toward a classification scheme of sedge (Cyperaceae) phytoliths. *In: Phytolith systematics*. Springer (Boston, MA): 91–111.
- Pearsall, D.M.**, 2015. *Paleoethnobotany: a handbook of procedures*. Left Coast Press (Walnut Creek, CA).
- Phumphumirat, W., Zetter, R., Hofmann, C.C. & Ferguson, D.K.**, 2015. Pollen degradation in mangrove sediments: a short-term experiment. *Review of Palaeobotany and Palynology* **221**: 106–116.
- Piperno, D.R.**, 1985. Phytolith analysis and tropical paleo-ecology: production and taxonomic significance of siliceous forms in new world plant domesticates and wild species. *Review of Palaeobotany and Palynology* **45**(3-4): 185–228.
- Piperno, D.R.**, 2006. *Phytoliths: a comprehensive guide for archaeologists and paleoecologists*. Rowman Altamira (Oxford).
- Piperno, D.R.**, 2014. *Phytolith analysis: an archaeological and geological perspective*. Elsevier (Amsterdam).
- Powers, A.H., Padmore, J. & Gilbertson, D.D.**, 1989. Studies of late prehistoric and modern opal phytoliths from coastal sand dunes and machair in north-west Britain. *Journal of Archaeological Science* **16**(1): 27–45.
- Ramírez, A.I.A., Rasbold, G.G., Parolin, M. & Stevaux, J.C.**, 2019. Phytoliths and seeds in fluvial island paleoenvironment reconstruction (interaction with pollen analysis). *Journal of South American Earth Sciences* **89**: 30–38.
- Ramsar Convention Bureau**, 1971. *Convention on Wetlands of International Importance especially as Waterfowl Habitat*.
- R Core Team**, 2013. *R: A language and environment for statistical computing [computer software]*, Vienna, Austria.
- Rovner, I.**, 1971. Potential of opal phytoliths for use in paleoecological reconstruction. *Quaternary Research* **1**(3): 343–359.
- Rovner, I.**, 1983. Plant opal phytolith analysis: major advances in archaeobotanical research. *In: Advances in archaeological method and theory*. Elsevier (Amsterdam): 225–266.
- Runge, F.**, 1999. The opal phytolith inventory of soils in central Africa—quantities, shapes, classification, and spectra. *Review of Palaeobotany and Palynology* **107**(1-2): 23–53.
- Schaminée, J.H.J., Sykora, K.V., Smits, N.A.C. & Horsthuis, M.**, 2010. *Veldgids plantengemeenschappen KNNV uitgeverij*.
- Scott, D.A. & Jones, T.A.**, 1995. Classification and inventory of wetlands: a global overview. *Vegetatio* **118**(1-2): 3–16.
- Shakoor, S.B., Bhat, M.A. & Mir, S.H.**, 2014. Phytoliths in plants: a review. *Research and Reviews: Journal of Botanical Sciences* **3**(3): 10–24.
- Team, R.**, 2015. *RStudio: integrated development for R*. RStudio, Inc. (Boston, MA): 639–640.
- van Geel, B., Coope, G.R. & van der Hammen, T.**, 1989. Palaeoecology and stratigraphy of the Lateglacial type section at Usselo (The Netherlands). *Review of Palaeobotany and Palynology* **60**(1-2): 25–129.
- Watling, J., Iriarte, J., Whitney, B.S., Consuelo, E., Mayle, F., Castro, W., Schaan, D. & Feldpausch, T.R.**, 2016. Differentiation of neotropical ecosystems by modern soil phytolith assemblages and its implications for palaeoenvironmental and archaeological reconstructions II: Southwestern Amazonian forests. *Review of Palaeobotany and Palynology* **226**: 30–43.
- Weng, C., Hooghiemstra, H. & Duivenvoorden, J.F.**, 2007. Response of pollen diversity to the climate-driven altitudinal shift of vegetation in the Colombian Andes. *Philosophical Transactions of the Royal Society B: Biological Sciences* **362**(1478): 253–262.
- Witteveen, N.H., Hobus, C.E.M., Philip, A., Piperno, D.R. & McMichael, C.N.H.**, 2022. The variability of Amazonian palm phytoliths. *Review of Palaeobotany and Palynology* **300**: 104613.
- Zhang, Y.J., Duo, L., Pang, Y.Z., Felde, V.A., Birks, H.H. & Birks, H.J.B.**, 2018. Modern pollen assemblages and their relationships to vegetation and climate in the Lhasa Valley, Tibetan Plateau. *China Quaternary International* **467**: 210–221.
- Zurro, D., García-Granero, J.J., Lancelotti, C. & Madella, M.**, 2016. Directions in current and future phytolith research. *Journal of Archaeological Science* **68**: 112–117.

Appendix A

The NMDS carried out over the concentrations showed a slightly different result. While samples of agricultural fields form a dense cluster again, it does overlap with wetlands on the first axis and forests on the second axis. Samples from forests are spread out over nearly the entire range of the second axis, thus having overlap with both other groups, but can all be found on the centre-left side of the first axis. Wetlands are even more distributed over the first axis and are the most distributed of all three groups, they can be seen on across the full range of the first axis and the complete bottom half of the second axis, thus having overlap with both other groups.

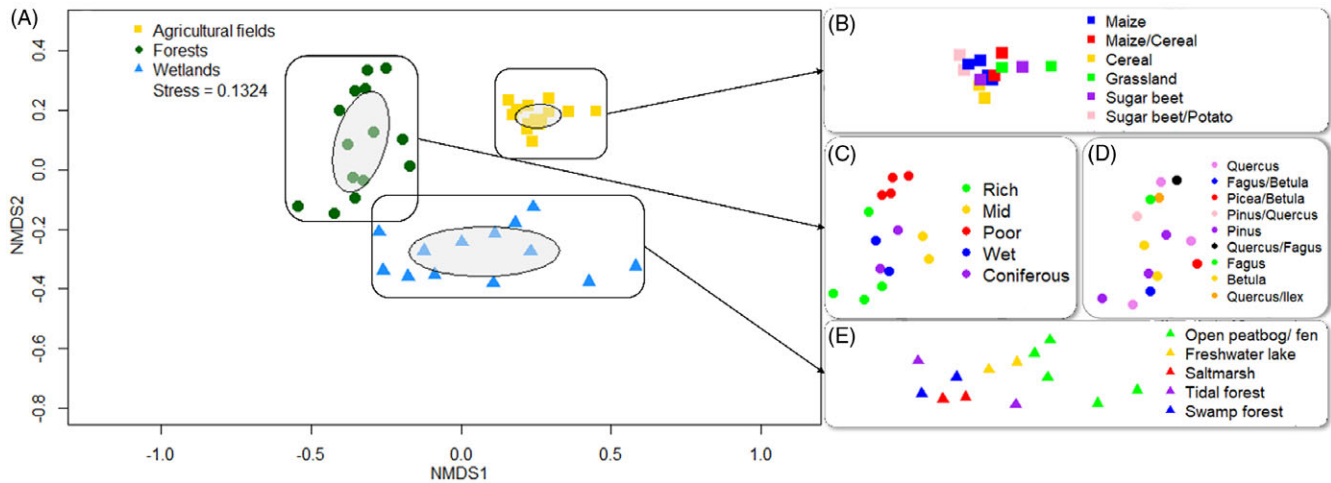


Fig. A1. NMDS of phytolith samples. The NMDS is carried out using the concentrations (phytoliths/1 cm³ soil) of each morphotype (stress = 0.1324). A: overall result of the NMDS, blue dots represent the samples taken from wetlands, the green dots the samples taken from forests and the yellow dots the samples taken from agricultural fields. The circles show the centre of the different clusters. B-E: within variation of the different vegetation types: B - agricultural fields, C - forests (forest type based on Schaminée et al. (2010)), D - forests (most commonly occurring tree species), E - wetlands.

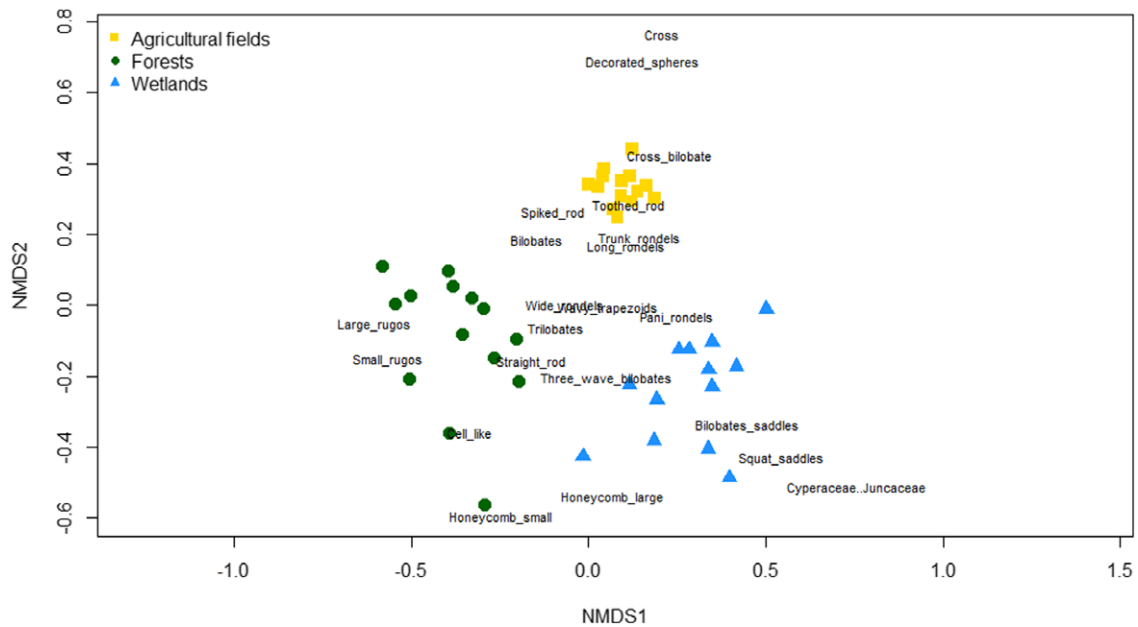


Fig. A2. NMDS with ordination scores of different phytolith morphotypes. NMDS carried out over the percentages of each morphotype (stress = 0.1459). Samples from agricultural fields are shown in grey squares, samples from wetlands in grey triangles. Samples from forests are categorised by the most commonly occurring tree species in the area.

Table A1. P-values of ANOVAs of the separate morphotypes. All extracted p-values from the ANOVAs (both percentages and concentrations) between the different vegetation types for each morphotype are shown.

Morphotype	Percentages (%)			Concentrations (# phytoliths/1 cm ³ soil)		
	Fo-Fi	Fi-We	Fo-We	Fo-Fi	Fi-We	Fo-We
Spheroid rugose (large)	<2E-16***	0.560 ^{n.s.}	<2E-16***	0.708 ^{n.s.}	0.836 ^{n.s.}	0.368 ^{n.s.}
Spheroid rugose (small)	<2E-16***	0.616 ^{n.s.}	<2E-16***	0.242 ^{n.s.}	0.393 ^{n.s.}	0.945 ^{n.s.}
Spheroid ornate	4.36E-06***	9.66E-07***	0.665 ^{n.s.}	5.55E-06***	1.26E-06***	0.665 ^{n.s.}
Total arboreal	<2E-16***	0.969 ^{n.s.}	<2E-16***	0.821 ^{n.s.}	0.567 ^{n.s.}	0.907 ^{n.s.}
Rondel (wide)	0.916 ^{n.s.}	0.581 ^{n.s.}	0.351 ^{n.s.}	0.021*	0.671 ^{n.s.}	0.003**
Rondel (elongated)	2.00E-07***	3.54E-04***	0.052 ^{n.s.}	1.26E-05***	0.366 ^{n.s.}	0.001**
Rondel (tabular)	<2E-16***	0.053 ^{n.s.}	<2E-16***	1.96E-05***	0.510 ^{n.s.}	8.00E-07***
Tent shaped body	1.21E-05***	0.005**	0.103 ^{n.s.}	3.21E-05***	0.422 ^{n.s.}	0.002**
Trapezoid	0.053 ^{n.s.}	0.889 ^{n.s.}	0.018*	0.006**	0.498 ^{n.s.}	2.25E-04***
Bilobate (thin castula)	0.008**	<2E-16***	8.51E-05***	0.010*	0.117 ^{n.s.}	0.583 ^{n.s.}
Bilobate (thick castula)	0.670 ^{n.s.}	0.446 ^{n.s.}	0.928 ^{n.s.}	0.016*	0.929 ^{n.s.}	0.045*
Polylobate (symmetrical)	0.754 ^{n.s.}	0.506 ^{n.s.}	0.170 ^{n.s.}	0.065 ^{n.s.}	0.652 ^{n.s.}	0.008**
Polylobate (asymmetrical)	0.979 ^{n.s.}	<2E-16***	<2E-16***	0.436 ^{n.s.}	5.03E-05***	1.10E-06***
Saddle	0.080*	<2E-16***	<2E-16***	0.018*	0.003**	1.34E-07***
Cross type 1 (small)	1.65E-06***	0.001**	0.136 ^{n.s.}	7.14E-06***	0.006**	0.077 ^{n.s.}
Cross type 1 (large)	<2E-16***	0.081 ^{n.s.}	4.60E-06***	0.002**	0.882 ^{n.s.}	0.001**
Total grasses	1.000 ^{n.s.}	1.01E-07***	2.02E-07***	1.000 ^{n.s.}	1.11E-07***	2.22E-07***
Papillate	1.10E-04***	0.044*	6.46E-05***	0.411 ^{n.s.}	2.55E-04***	0.009**
Asteraceae	0.010*	6.35E-4*	0.205 ^{n.s.}	0.733 ^{n.s.}	0.693 ^{n.s.}	0.998 ^{n.s.}
Spheroid psilate	2.93E-06***	2.20E-06***	0.001**	1.76E-04***	0.177 ^{n.s.}	0.022*
Elongate entire	0.009**	0.008**	0.048*	0.011*	0.038*	0.510 ^{n.s.}
Elongate dentate	0.002**	0.012*	0.003**	0.002**	0.034*	0.538 ^{n.s.}
Elongate dendritic	0.629 ^{n.s.}	0.001**	8.91E-05***	0.001**	0.027*	3.00E-07***
Total herbaceous				0.018*	0.736 ^{n.s.}	0.003**

Abbreviations: Fo-Fi: forests vs. fields; Fi-We: fields vs. wetlands; Fo-We: forests vs. wetlands.

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ^{n.s.} $p > 0.05$.

Table A2. Mean \pm SD of the percentages and concentrations of the separate phytolith morphotypes. All extracted means and standard deviations (both percentages and concentrations) of each morphotype are shown.

Morphotype	Percentages (%)			Concentrations (# phytoliths/1 cm ³ soil)		
	Forests	Fields	Wetlands	Forests	Fields	Wetlands
Spheroid rugose (large)	9.8 \pm 2.8	2.9 \pm 1.0	2.1 \pm 1.9	18084 \pm 15697	16758 \pm 23828	12615 \pm 14412
Spheroid rugose (small)	18.8 \pm 3.7	4.0 \pm 0.9	5.9 \pm 4.7	36839 \pm 35143	23754 \pm 32226	36381 \pm 42671
Spheroid ornate	0.1 \pm 0.2	1.8 \pm 0.7	0.1 \pm 0.1	229 \pm 611	8921 \pm 9305	215 \pm 552
Total arboreal	31.3 \pm 5.8	9.9 \pm 1.9	10.4 \pm 6.6	59399 \pm 52587	55977 \pm 73313	112851 \pm 243291
Rondel (wide)	31.1 \pm 4.8	31.7 \pm 3.8	33.5 \pm 5.1	56730 \pm 45062	164930 \pm 165690	651210 \pm 1721452
Rondel (elongated)	1.2 \pm 0.8	3.6 \pm 1.0	2.0 \pm 0.9	1901 \pm 1561	18354 \pm 18949	45383 \pm 132302
Rondel (tabular)	1.4 \pm 1.1	3.9 \pm 0.9	2.2 \pm 1.4	2024 \pm 2061	21181 \pm 22599	45187 \pm 117952
Tent shaped body	1.3 \pm 1.9	3.4 \pm 0.7	4.3 \pm 1.0	2127 \pm 3192	17788 \pm 19868	85848 \pm 233960
Trapezoid	12.1 \pm 5.0	17.6 \pm 3.7	18.7 \pm 8.6	20427 \pm 15382	89661 \pm 90339	406210 \pm 1174653
Bilobate (thin castula)	12.4 \pm 3.7	16.9 \pm 4.2	7.1 \pm 5.2	22842 \pm 20510	103369 \pm 135229	201918 \pm 577181
Bilobate (thick castula)	0.4 \pm 0.4	0.3 \pm 0.4	2.0 \pm 0.8	557 \pm 584	2219 \pm 3641	42662 \pm 117357
Polylobate (symmetrical)	2.5 \pm 2.4	2.4 \pm 0.8	2.3 \pm 1.7	3694 \pm 2722	14398 \pm 18206	97106 \pm 326759
Polylobate (asymmetrical)	0.6 \pm 0.7	0.7 \pm 0.6	1.0 \pm 0.6	684 \pm 854	4657 \pm 9067	27848 \pm 88736
Saddle	0.4 \pm 0.6	0.6 \pm 0.3	4.6 \pm 1.9	367 \pm 487	3494 \pm 4367	49614 \pm 99836
Cross type 1 (small)	1.1E-3 \pm 2.0E-3	9.0E-3 \pm 4.4E-3	3.0E-3 \pm 2.9E-3	7 \pm 12	60 \pm 39	46 \pm 63
Cross type 1 (large)	1.8E-4 \pm 6.6E-4	0.02 \pm 0.01	0.0 \pm 0.0	2 \pm 6	78 \pm 48	0 \pm 0
Total grasses	63.5 \pm 7.0	83.5 \pm 2.4	78.0 \pm 9.0	111598 \pm 83546	453243 \pm 487967	1658745 \pm 4596491
Papillate	0.0 \pm 0.0	0.0 \pm 0.0	6.1 \pm 2.9	0 \pm 0	0 \pm 0	117611 \pm 321689
Asteraceae	0.9 \pm 0.5	0.1 \pm 0.2	2.2 \pm 2.1	1185 \pm 1689	932 \pm 1642	15979 \pm 29236
Spheroid psilate	2.3 \pm 2.0	0.7 \pm 0.5	1.1 \pm 1.7	3018 \pm 2542	2447 \pm 2431	4151 \pm 4930
Elongate entire	2.7 \pm 1.8	1.2 \pm 0.9	2.4 \pm 1.0	4247 \pm 3236	6544 \pm 9550	63640 \pm 191419
Elongate dentate	0.8 \pm 0.8	3.3 \pm 1.5	1.2 \pm 0.7	1314 \pm 1480	16192 \pm 23713	14135 \pm 28543
Elongate dendritic	1.2 \pm 0.8	2.4 \pm 1.5	1.0 \pm 1.0	2070 \pm 1972	10546 \pm 10541	16101 \pm 43941
Total herbaceous	5.2 \pm 3.0	6.5 \pm 2.2	11.6 \pm 5.1	8005 \pm 5952	30117 \pm 31893	169465 \pm 419972
Total				179001 \pm 137775	539337 \pm 588073	1941062 \pm 5257445