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REPRESENTATIONAL BIAS IN PHYTOLITHS FROM MODERN SOILS OF CENTRAL NORTH AMERICA: IMPLICATIONS FOR PALEOVEGETATION RECONSTRUCTIONS

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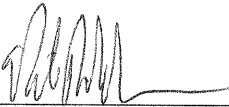
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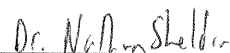
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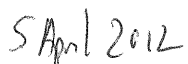


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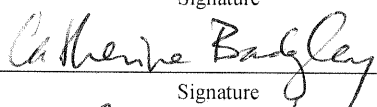
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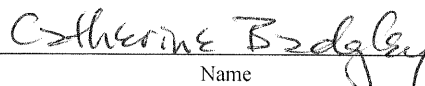
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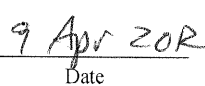
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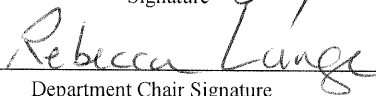
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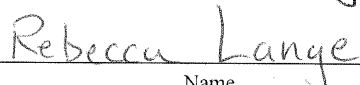
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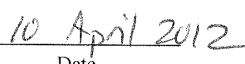
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REPRESENTATIONAL BIAS IN PHYTOLITHS FROM MODERN SOILS OF CENTRAL
NORTH AMERICA: IMPLICATIONS FOR PALEOVEGETATION RECONSTRUCTIONS

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ABSTRACT

Understanding localized patterns and community compositions of vegetation in an environment is critical to the reconstruction of climatic and ecological conditions across all spatiotemporal scales. One of the most accurate and useful ways to characterize vegetation, and therefore to describe the climatic and ecological conditions of a location, is through the plant fossil record. Phytoliths (plant silica microfossils) are often preserved in the absence of other paleobotanical data and are becoming more widely used for deep-time vegetation reconstructions. Significant work has been done to standardize the analytical methodology of phytolith extraction, statistical analysis, and interpretation, but more detailed investigations are needed to understand how well a given phytolith assemblage represents the aboveground plant biomass for a given ecosystem.

We present results from paired soil phytolith assemblages and local vegetation assemblages across the central United States, from temperate forest, grassland, and rangeland/scrubland ecosystems. Phytolith assemblages from soil A-horizons were compared to percent cover of species and plant biomass estimates obtained via field observations and aerial estimates of tree cover to analyze differences in the relative abundance of forest or woody vegetation versus grasses. Soil phytolith assemblages from all sites average a 32% bias toward the grass morphotypes as compared to actual aboveground biomass observations, and comparisons to percent cover yielded broadly comparable bias figures. Percent bias estimates do not show significant correlations to most environmental factors (temperature, precipitation, local elevation), however, an extremely strong correlation ($p < 0.001$) was observed with soil order type. As a result, we suggest further research into the development of correction factors between phytolith sample assemblages and their inferred past counterpart ecosystems based on estimates derived from modern analyses of each major soil order type. Such corrections are essential to the

continued use of phytoliths as a proxy for past vegetation and ecological reconstructions throughout the Phanerozoic record.

KEYWORDS: phytolith, taphonomy, soil, vegetation

1. INTRODUCTION

Climate change and its future impacts have been a major focus of recent scientific investigation. Using records of past episodes of climatic change and their resulting impacts on biological systems provides an important analogue for predicting current and future change and for describing the links between climate and ecology. To this end, it is crucial that we develop methods of tracking vegetation histories and of quantifying ecological change as a function of climate.

Traditionally, records explaining the relationship between vegetation and climate have been assembled using pollen cores (e.g., Adams et al., 1990; Jackson and Williams, 2004; Hatté et al., 2008; Jaramillo et al., 2010; Liu et al., 2010), and much work has been done to standardize palynological methods and their implications with regard to reconstructing periods of climate change (e.g., Erdtman, 1943; Davis, 1968; Moore et al., 1991; Blois et al., 2011). However, due to a dearth of suitable sites (typically lakes) for the deposition and preservation of pollen in central North America and other continental interiors, palynological studies have remained fragmentary for records older than the Quaternary. Consequently, descriptions of vegetation history have poor or uneven spatial and temporal resolution for most continental interiors. While the availability of lacustrine cores is limited, soil records in many temperate continental interiors are both spatially and temporally more robust, allowing for the detailed description of past and present vegetation through the use of phytolith assemblages.

1.1 Phytoliths

Phytoliths are silica or bio-opal microfossils that form in the interstices or lacunae of plant cells as a result of plants' inability to assimilate into their tissue the silica in the monosilicic acid (H_4SiO_4) that they uptake from groundwater solutions (Piperno, 1988; Piperno, 2006).

Many plants deposit a hydrous (opalline) form of silica either in between dermal cells or as a coating on tracheary or structural elements, which provides support, rigidity, and structural defense mechanisms for the plant (Jacobs et al., 1999; Piperno, 2006). Plant taxa, having disparate internal structures and in some cases exhibiting preferential modes of silica deposition, can have specific morphotypes for these deposits, meaning that phytoliths can be used to identify different plant types at various taxonomic levels (e.g., Piperno, 2006). Phytoliths have been identified from many vascular plant groups, including most angiosperm clades, conifers, and ferns (Piperno, 2006), and have been described for some groups as far back as the Devonian in both sediments and coprolites (e.g., Carter, 1999). Significant work has been done on the extraction methodology and on applications of phytoliths to climatic and ecological questions (e.g., Rovner, 1971; Piperno, 1988; Piperno and Pearsall, 1993; Strömberg, 2002, 2004). Due to the climatic dependence of plants, phytolith assemblages have been used as a proxy for understanding climate characteristics and local climate impacts on vegetation (e.g., Thomasson, 1990; Fredlund and Tieszen, 1997; Barboni et al., 1999). Phytoliths have also been applied as a method of tracking the evolution of metabolic pathways and species divergences, as many plant groups conserve basic phytolith morphologies through time (Smith, 1996; Smith and White, 2004).

Methodology studies of modern phytoliths have addressed many of the taphonomic concerns about the representativeness of assemblages. Works by Piperno (1988, 2006) and Piperno and Pearsall (1993) have shown that phytoliths are easily incorporated into soils upon plant death and can provide an accurate *in situ* record of specific vegetation types and climatic conditions even for small scale variation across a catena. Additionally, the fact that phytoliths for nearly all studied plant species are of similar size (2–250 μm) and weathering-resistant

composition ($\text{SiO}_2 \cdot n\text{H}_2\text{O}$) minimizes potential preservation biases seen in other terrestrial records such as macrofossils and palynomorphs (Baker, 1959). The occurrence of phytoliths in most vascular plant taxa and throughout the lifecycle of an individual means that in many cases, preservation potential or the probability of representation in the phytolith record is extremely high. Other taphonomic issues have been raised under certain circumstances: 1) limited fluvial and aeolian sorting of phytoliths based on shape and specific gravity has been demonstrated in high-energy systems like river channels or dune fields (Lawlor, 1995); and 2) slight representational skewing from transport and selective destruction is evident in situations of intense herbivory (Fredlund and Tieszen, 1994). In most cases however, these issues can be discounted as factors influencing assemblage composition due to their limited applicability. Further complications in terms of quantitative reconstructions come from multiplicity and redundancy within phytolith morphotypes (Rovner, 1971); however, exhaustive modern reference collections have begun to address these factors (e.g., Strömberg, 2003; Piperno, 2006; Pearsall, 2011; Pereira, 2011).

1.2 Problems with the record

Despite significant work on extraction methods and taphonomic biases, little has been done to evaluate the accuracy of relative species representation in the soil phytolith record (Table 1), especially in temperate ecosystems. However, it has been noted that some North American temperate zones produce phytolith assemblages that are less reliable for ecosystem inferences than similar records from the tropics (Strömberg, 2004). Differences in biomass production rates and silica uptake among plant types impact their proclivity to produce phytoliths, resulting in potential biases towards some taxa and against others (Piperno, 1988). For example, grasses produce prolific diagnostic phytoliths, while hardwood trees produce very few diagnostic

phytoliths (Piperno, 2006) in total assemblages from soil samples; as a result, we might expect an over-representation of grass groups. Additionally, some work suggests that certain depositional environments, including some soil types, may be unsuitable for phytolith preservation due to oxidization or clay-adhesion (Fredlund and Tieszen, 1997).

These factors are problematic for the interpretation of environmental conditions based on relative percentages of phytolith assemblages from paleosols (e.g., Strömberg, 2002, 2004, 2005; Strömberg et al., 2007), as it is unclear whether these assemblages are accurate representations of their original ecosystems. Paleovegetation reconstructions using phytoliths preserved in paleosols are done by counting phytolith morphotypes and classifying them under plant-type categories (e.g., forest or woody plant, grasses, aquatic plants) based on analysis of a broad, non-region-specific modern reference collections and published work (Strömberg, 2003, 2005, 2007; Zucol, 2010; Smith, unpublished data), since for fossil assemblages we do not know a priori what species were present. While many studies of modern phytoliths (e.g., Carnelli et al., 2001; Tsartsidou et al., 2007) have compared assemblages with phytolith extractions from local standing vegetation, the assumption that a soil phytolith assemblage is representative of standing vegetation biomass has not been verified. Recent studies by Carnelli et al. (2001), Lu and Liu (2003), and Blinnikov (2005) have suggested that any quantitative paleoenvironmental reconstruction using phytoliths must begin with analyzing modern phytolith distribution and representation in local plants and soils, but to date none have been completed for central North America or other temperate continental interiors. The aim of this study is to test how accurately standard methods of paleovegetation inference from phytolith assemblages reflect vegetation types (woody or forest plants versus grasses) by applying it to modern localities with known vegetation composition. We utilized traditional phytolith extraction and analysis and

aboveground biomass estimate techniques to quantify production, preservation, and environmental biases in modern soil phytolith assemblages and to suggest calibrations for ecosystem-specific correction factors. These calibration results were then used in a case study to reinterpret fossil phytolith assemblage data from a continental-interior site in South America.

2. METHODS

2.1 Sites

Locations in this study include both forest (Alfisol and Spodosol soil types) and grass/rangeland (Mollisol and Inceptisol soil types) ecosystems, which are the primary ecotypes of the central United States (FIG. 1; Table 2). Sites within these ecotypes represent a range of geographic (FIG. 1) and climatic (Table 2) conditions, in an effort to constrain the impacts of these variables on assemblage composition and bias. Each site was chosen based on known soil series types as described by the Natural Resources Conservation Service Soil Survey (2011), and in each case was from a protected area or research preserve representing primary ecosystems with no history of significant human alteration, in an attempt to exclude potential complications from phytolith assemblage inheritance (e.g., Fredlund and Tieszen, 1994). Forest sites (n= 11) were characterized by soil type, classified as either Alfisols or Spodosols, and by aboveground biomass estimates of >50% woody dicotyledonous species. Such sites included Oak Savannah, Northern Hardwood, Laurentian Coniferous, Coastal Plain Deciduous, Southern Oak-Hickory, and Eastern Broadleaf forests (Bailey, 1995). Grass/rangeland sites (n= 7) were also characterized by soil type, classified as either Mollisols or Inceptisols, and by aboveground biomass estimates of <50% woody dicotyledonous species (primarily monocot/grass species). Such sites included both Prairie Parkland and Great Plains ecosystem provinces (Bailey, 1995).

Individual sites were identified based on the Intensive Plot model (Barnett and Stohlgren, 2003), with a larger central sampling plot (2m x 5m) and four smaller sampling plots (1m x 1m) located randomly within the overall site (10m X 20m). Soil cores and vegetation samples were taken from within each of the sampling plots, and were characterized qualitatively across the rest of the site.

2.2 *Vegetation*

For the sampled sites, total biomass was derived from diameter at breast height measurements (DBH) of all tree diameters >10 cm, and all other species were measured within sampling plots to full standing height (Zak et al., 1989). Biomass was estimated as a function of percent ground cover and basal area (e.g., Röttgermann et al., 2000; Barnett and Stohlgren, 2003; Suchar and Crookston, 2010) with an assumed standard error of $\pm 5\%$. In addition to ground-based estimates of total biomass, we compiled aerial estimates of percent cover (e.g., Roy and Ravan, 1996; Barboni et al., 2007) for each site. Mean tree-cover data were derived from the Moderate Resolution Imaging Spectroradiometer (MODIS) visible bands and Normalized Difference Vegetation Index (NDVI), which provide estimates for the proportion of tree cover for any given 500×500 m sample of Earth's surface (Hansen et al., 2005). MODIS percent tree cover corresponds to the amount of skylight obstructed by tree canopies ≥ 5 m in height, and values are averaged over one year to avoid cloud interference and phenological variation in tree cover (Hansen et al., 2003; 2005).

To test best-fit methods for vegetation interpretations we compared soil phytolith assemblage counts to both ground-based biomass estimates (e.g., Zak et al., 1989) and aerial percent-cover estimates (e.g., Hansen et al., 2003, 2005). These methods of estimating vegetation composition utilize fundamentally different metrics for describing vegetation

characteristics, as biomass is a mass per unit area, while cover is a ratio of area measurements. Ultimately, most modern studies employ some combination of the two, whereas many paleovegetation reconstructions simply use inferred cover estimates to determine whether a site's vegetation was open or closed, or how much of the ground surface was shaded (e.g., Strömberg, 2005). We chose to compare the two methods here by converting both to a percentage of the total vegetation (% biomass and % cover).

2.3 *Soil phytoliths*

Strömberg (2004) proposed a synthetic analytical approach for Cenozoic phytolith assemblages, involving the study of phytoliths from all size fractions (2–250 μm), the comparison of morphotype relative frequencies over time including non-diagnostic and ecologically significant (e.g., aquatic) forms, and the broader generalization of correlations between assemblage composition and vegetation structure (forest vs. grassland indicators, instead of specific analogues). This broad approach was refined based on a compilation of many different studies and comparison to a large modern reference collection (this study; Strömberg, 2003, 2004, 2005; Smith, unpublished data) in an attempt to standardize methodology across all time scales. The extraction methodology used here was slightly modified from Strömberg et al. (2007) for the study of modern soil types.

Sampling involved systematic coring of soil A-horizons without litter layers at multiple locations within sampling plots at each site with a 125 cm^3 push core from the upper 5–10 cm. These samples were homogenized, and small subsamples (<5 g) of unsieved sediment were processed with 10% hydrochloric acid to remove carbonates, and wet oxidized in 70% nitric acid (HNO_3) and potassium chlorate (KClO_3) to remove organic material. Coarse material was removed with a 250 μm mesh sieve, and samples were deflocculated by sieving through a 53 μm

mesh sieve. Fractions were then recombined before being gravimetrically separated via heavy liquid (ZnBr_2) flotation at a density of 2.38 g cm^{-3} . The resulting float material was washed in ethanol and dried before being mounted on slides in immersion oil to examine shapes under rotation. Morphotypes were then counted in linear, cross-slide transects and photographed with a Leica petrographic microscope (400–1000X) on slides prepared with Cargille Meltmount 1.539. Over 200 diagnostic individuals were counted per slide and all morphotypes were identified under the classification system of Strömberg (2003, 2005), except for Ho-1 (hollow, thick-walled spherical morphotype found in woody plants; FIG. 2) that is recognized based upon the work of Bozarth (1992), and are detailed in Supplementary Data. Calculation of total Forest Indicator (FI) and Grassland Indicator (GI) morphotypes followed the descriptions of Strömberg (2003), where FI total is the sum of dicotyledons, general forest indicators, conifers, non-grass plants, palms, and Zingiberales, and GI is the sum of all grass silica short cells (GSSCs) and diagnostic grass phytoliths (GRASS-D). While some previous authors have excluded GRASS-D morphotypes because of the possibility that their abundance is tied to moisture availability (e.g., Bremond et al., 2002; Strömberg, 2003), we find only a very weak relationship between moisture availability and GRASS-D abundance in our dataset (FIG. S1), and therefore include them here as an important component of the total phytolith assemblage.

In an effort to consider possible sources of experimental design bias, we evaluated results related to laboratory techniques, statistical treatments, and interpretation methodologies. Phytolith counting yield was included in our independent variable list as a method for analyzing potential biases stemming from current extraction techniques (e.g., Piperno and Pearsall, 1993; Strömberg, 2002). Accepted counting error for assemblages is given at 7.7% for all phytolith groups (e.g., Strömberg, 2002), but this value is based on the standard deviation of multiple

count experiments with morphotype classifications that are more complicated than our FI/GI distinction. For the purposes of describing and comparing ecosystem types in the most statistically appropriate manner for paleovegetation reconstructions, we can minimize assemblage counting error by collapsing these morphotype groups into FI and GI functional groups and performing a repeated measures analysis, which provides a specific error value for any individual recounted samples and results in a much smaller counting error function of ~1% for this suite of samples. Such recounts, when performed on selected assemblages, can significantly decrease experimental error from counting.

2.4 Statistical analyses

Statistical analyses were performed on phytolith assemblage counts based on ecosystem type categories established in the modern reference collections of Strömberg (2003, 2004, 2005). The relative populations of these phytolith assemblage categories were compared to aboveground biomass estimates for equivalent vegetation categories, and any significant differences ($> 1\sigma$) were considered examples of representational bias. Other work (e.g., Alexandre et al., 1997; Strömberg, 2002) has used the strict *d:p* (dicotyledon : grass phytolith) metric as an analytical tool for comparing forest and grassland indicators. Here we avoided this method due to the fact that it was developed specifically for modern tropical forest environments, and has been shown to be uncharacteristic of temperate ecosystems in the present and many ecosystems in the past (Strömberg, 2004), which is problematic for the intended applications of this study.

For individual sites where representational bias was evident, we performed linear regressions and analysis of variance (ANOVA) tests in the Statistical Package for Social Scientists (SPSS; IBM Corporation, 2011) comparing observed bias and a suite of environmental

variables (Table 2), including: soil order type, soil texture, sample count yield, site elevation, mean annual temperature (MAT), mean annual precipitation (MAP), and effective energy and mass transfer (EEMT; Rasmussen and Tabor, 2007). Where bias had strong correlation with this suite of environmental variables, correction factors for phytolith assemblages were devised based on the mean bias values for each category. In addition to analyzing the relationship between bias magnitude and potential causal variables, we used repeated measures analysis in SPSS (IBM Corporation, 2011) to estimate a standard error between assemblage categories for recounting individual sample yields. This is intended to assign a numerical counting error for assemblages at individual sites.

3. RESULTS

3.1 Aboveground Vegetation

A total of 35 plant genera were found across all study sites, and each genus was categorized as either a “Forest Indicator” or “Grassland Indicator” based on ecotype affinity. This total included 17 genera of Forest Indicators (FI), and 18 genera of Grassland Indicators (GI), with an average of 4 FI and 3 GI groups observed per individual site. Aboveground vegetation biomass estimates from ground plots for all sites ranged from 0% to 96% FI biomass, averaging 50.8% ($\sigma = 40.5\%$) of the total aboveground vegetation biomass (Table S1). For aerial estimates of vegetation type, tree cover estimates from MODIS data were considered percent FI for individual sites. Estimates of FI cover ranged from 0 to 100%, averaging 49.8% ($\sigma = 44.6\%$) of vegetation present (Table S1).

3.2 *Phytolith assemblages*

Soil bio-silica assemblages included 21 diagnostic phytolith morphotypes (FIG. 2) and 6 non-diagnostic phytolith morphotypes, as well as other non-phytolith groups (including diatoms and unidentifiable silica bodies; Table S2). Diagnostic morphotypes were categorized as either “Grassland Indicator”, which combined the GRASS, PACCAD, POOID, and CHLOR compound variables of Strömberg (2003, 2005; Table S2), or “Forest Indicator”, which combined the CONI, FI-GEN, and DICOT compound variables of Strömberg (2003, 2005; Table S2). Non-diagnostic and Other groups were excluded from assemblage analyses. This is a subset of the total compound variables used by Strömberg (2003, 2005), reflecting only those morphotypes found in our current study sites. The overall assemblage from all sites included 8 Forest Indicator (FI) morphotypes and 13 Grassland Indicator (GI) morphotypes, with an average of 4 FI and 8 GI morphotypes observed at each individual site. Sites studied had from 1.1% to 71.2% FI morphotypes, averaging 15.2% ($\sigma = 16.2\%$) of the diagnostic phytolith assemblage (Table S2).

3.3 *Representational bias*

Based on the comparison of aboveground vegetation and phytolith assemblages for individual sites, it is clear that representational bias exists in the phytolith record (FIG. 3). Representational bias is defined as the difference between %FI biomass and %FI morphotypes for an individual site. Bias estimates for the sites ranged from 0% to 72%, with an average of 32.2% bias ($\sigma = 32.3\%$) between observed biomass and phytolith estimates (FIGS. 3 and 4; Table S2).

Bias estimates were regressed against the environmental variables of site elevation, mean annual temperature (MAT), mean annual precipitation (MAP), and the combined metric effective energy and mass transfer (EEMT), and were analyzed for variance (ANOVA) for the categorical

variables of soil order type, soil texture, experimental yield. These regressions resulted in non-significant linear relationships between all climatic factors (FIG. 5). Soil order type was the only categorical variable found to have statistically significant mean differences in ANOVA tests ($p < 0.001$), indicating an important correlation between bias values and soil order type (FIG. 5C). Soil texture showed near-significant variance ($p = 0.105$), but all other variables failed this significance test.

3.4 *Multiple counts*

Recounted samples ($n = 9$) from each soil order type displayed broadly similar results in terms of morphotype group distinctions and specific morphotype counts within groups (Table S2). Repeated measures analysis found all recount samples to be statistically indistinguishable for ecosystem type categories (FI and GI; Table S2). Therefore, using standard extraction practices and counting methods in paleovegetation analyses, we can assign an overall counting error of 1.1% (0.5–2.1%) for these phytolith analyses based on 1σ values.

4. DISCUSSION

4.1 *Phytolith record bias*

While significant work has been done to establish common extraction, statistical treatment, and environmental interpretation methods (e.g., Strömberg, 2002, 2004, 2005), many recent studies have highlighted problems with suspected bias between actual aboveground vegetation and soil phytolith samples (Table 1). In light of our results, we suggest major revisions to certain aspects of this process, particularly in the realm of how past environments are inferred and compared using phytolith abundance data. This study has many implications for the

treatment of future phytolith data, both in terms of methodology and error reporting and in terms of the interpretation of phytolith assemblage data.

4.1.1 Method testing

We tested both phytolith and vegetation estimation methods, and found both common techniques to be robust. Based on the lack of correlation between discernible bias values and phytolith yield (FIG. 5A), we concluded that common extraction procedures (e.g., Strömberg et al., 2007) are appropriate for this system and are not responsible for introducing systematic bias into phytolith assemblages. Additionally, the generally predictable 1:1 linear relationship between ground-based biomass estimates and aerial percent cover estimates (Table S1; e.g., Muukkonen et al., 2006) allows us in most cases to use these two metrics interchangeably, making either of them applicable to the types of paleovegetation studies with which we are concerned. While the percent biomass and percent cover values are broadly similar in our analysis (Table S1), there is an apparent skewing toward slightly higher percent cover values relative to percent biomass values, probably resulting from the documented inability of aerial methods to resolve the presence of understory vegetation (Hansen et al., 2005). For this reason, we have focused our interpretations on bias comparisons based on estimated percent biomass values.

4.1.2 Soil order type

Possibly our most important result is the strong correlation between phytolith assemblage bias and soil order type (FIG. 5C). This was the only significant correlation found between our described factors ($p < 0.001$), and the correlation explains nearly all of the variability in bias values. The only other analyzed factor that appeared to explain any of the variability was soil texture, which happens to be intricately linked with soil order due to the processes of soil

development (Brady and Weil, 1996; Retallack, 2001), and likely covaries with any assignment of a soil order variable.

The correlation to soil order not only provides an important linkage between observed assemblage bias (Table 1; this study) and a defined environmental factor, it provides a potential treatment (in the form of a correction factor) for phytolith assemblage data for cases in which the soil order type is well described. Our data cluster into three distinct groups (FIGS. 4 and 5), where average Alfisol/Spodosol bias (excluding one site, discussed below) is 62.1% ($\sigma = 5\%$), average Inceptisol bias is 6% ($\sigma = 1\%$), and average Mollisol bias is 1.3% ($\sigma = 1.2\%$). Alfisol and Spodosol groups were combined due to their functional similarities among modern soils and because they are collapsed into a single order for most paleosol analyses (e.g., Mack et al., 1993) since the geological record of Spodosols is much more limited than for Alfisols. A single site was excluded from the Alfisol/Spodosol type average due to its status as an outlier ($>7\sigma$), which was likely the result of gathering soil cores in areas of high-density *Acer* saplings, known to be significant phytolith producers (Bozarth, 1992). Excepting this site, all of the soil order groupings have bias averages that are well-defined by small standard deviations, allowing us to propose the use of these average values as correction factors for each of these major soil orders. While these correction factors do not allow us to disentangle the relative contributions of production and taphonomic biases, they do allow us to correct for the combined influence of such bias on environmental interpretations.

These biases have significant implications for interpreting past environments, as most work on describing environmental change using phytoliths (e.g., Strömberg, 2005; Zucol et al., 2010) relies on the extraction of phytolith assemblages from these soil order types, and most paleoecological reconstructions using paleosols come from these three soil orders (Retallack,

2001). By assigning correction factors for each of these soil orders, we can compensate for observed differences between aboveground biomass (which determines whether vegetation is classified as “forest” or “grassland”) and the resulting phytolith assemblage, standardizing the way paleoenvironmental interpretations are made in a similar manner as other fields such as palynology (e.g., Baker, 1959). The use of correction factors is crucial for the application of phytoliths as a paleoenvironmental indicator, and the direct interpretation of phytolith assemblages should be reconsidered in light of our results. Experiments designed to develop interpretational correction factors should also be repeated for the range of other soil orders (Ultisols, Oxisols, Vertisols, etc.) that can be accommodated by paleoenvironmental reconstructions.

4.1.3 Other factors

Many other environmental and climatic factors (e.g., MAP, MAT, EEMT, elevation) have been suggested as potential sources of bias by previous work (Table 1). Based on our results, all of these factors appear to be non-significant, as none show correlations or linear relationships with bias variability in any of our sites (FIG. 5). These factors are also all tightly linked, as MAT and MAP (as well as EEMT) are strongly influenced both by each other and by elevation, and suggested relationships or observations of similarity between these factors and phytolith bias from some locations (e.g., Barboni et al., 2007; Iriarte and Paz, 2009), may actually be due to differences in soil order. Soil order may be the best estimate of total bias in the phytolith record, as it is a function of many factors (climate, environmental conditions, time, vegetation/ecosystem type; Retallack, 2001), and likely aggregates both production and taphonomic influences. While elevation is statistically insignificant for our current regressions, it

is worth noting that our dataset includes very few intermediate (500–1500m) elevation sites; thus, further sampling may reveal elevation as a potentially useful indicator for bias estimates.

4.2 Case study: Zucol et al.(2010)

A recent study by Zucol et al. (2010) is an excellent example of commonly used practices in the use of phytoliths as an indicator of environment type and method for tracking the evolution of grasslands. The work examines a stratigraphic section in southern Argentina (Gran Barranca) from the Eocene that has been described as mixed pyroclastics and paleosols with significant depositional hiatuses and periods of pedogenesis (Bellosi, 2010). Phytoliths were extracted from paleosols in this section via methods of Zucol et al. (2010), which have been compared favorably with other standard methods (Strömberg, 2011). The described phytolith assemblage has 33 morphotypes divided into grass and non-grass (palms, herbaceous monocots, dicots, and others) groups. These groups are then plotted as percent abundances through time, without quantitative error estimates, and interpreted directly as relative vegetation abundances in the paleoenvironment (FIG. 6). Zucol et al. (2010) concluded that these abundances indicate the early (Eocene) presence of significant grassland ecosystems (>50% grass-type vegetation) in southern Argentina, including a measurable C₄ grass component. This interpretation is a novel result, as vegetation inferred from other Paleogene locations throughout the globe has indicated that the evolution and spread of grasslands occurred significantly later (e.g., Jacobs et al., 1999; Strömberg, 2005; Edwards et al., 2010), and that C₄ grasses were not a major component of such ecosystems until the Miocene (~8–0 Ma ago; Latorre et al., 1997; Jacobs et al., 1999; Fox and Koch, 2004; Strömberg, 2011; Strömberg and McInerney, 2011).

The work by Zucol et al. (2010) is a common example of the way phytoliths are employed in paleoenvironmental reconstructions, which is why we find it necessary to examine

some of the assumptions made by this model in light of our findings about sources of bias in such analyses. Zucol et al.'s (2010) use of standard phytolith extraction and age correlation (sampling from known dated levels within a constrained outcrop) are appropriate, though the lack of any reported quantification of counting or experimental error is problematic, as it is unclear that any sample recounts were undertaken. While errors between recounts are likely minimal (~1% in our error analysis), error reporting in phytolith counting is generally lacking and should be improved community-wide (Blinnikov, 2005).

Phytolith assemblages are an important tool for reconstructions like that of Zucol et al. (2010), but as with all proxy data, they require the use of other types of data for context. In this case, sedimentological descriptions or pedotype classifications for the sampled horizons, or stable isotope stratigraphy could greatly improve any interpreted changes and provide stronger ties between data and interpretations on the whole. While pedotypes (soil orders) are not defined for this work, or for the stratigraphic descriptions of Bellosi (2010), descriptions of paleosols containing thick clay and carbonate horizons (Bellosi, 2010) as well as defined phytolith zonation within paleosols (Zucol et al., 2010) suggest that the site contains paleosols fitting a modern description of Inceptisol and Alfisol. Lack of mollic features or any observed organic carbon preservation, as well as the age of the section, make a Mollisol classification unlikely, calling into question the group's interpretation that this environment supported significant (>50%) grassland vegetation.

In the context of our current study, the lack of supporting environmental data, and the likelihood that soil order types for the phytolith levels reported in Zucol et al. (2010) are analogous to modern Inceptisols and Alfisols suggests that the direct interpretation of phytolith assemblage data was erroneous. When we correct the raw assemblage data from this site using

the factors defined by mean bias values for each pedotype, the new results have minimal grassland indicators (FIG. 6). Additionally, the inclusion of a C₄ component to the grass assemblage should be reviewed, as the taxon (Panicoideae) assigned to the “C₄” component contains both C₃ and C₄ groups (Strömberg, 2011). Revising the paleoenvironmental interpretation of the Gran Barranca site to instead include the limited presence of grassland indicators fits more closely with other interpretations of the region (Barreda and Palazzesi, 2010; Bellosi, 2010) and the general pattern of worldwide grassland evolution (e.g., Strömberg, 2005, 2011; Strömberg et al., 2007; Strömberg and McInerney, 2011), which are consistent with grasses being sparse and the Eocene of South America being a generally forested landscape. Therefore, applying this correction is an important step forward in reconciling the floral records of such an important locality. This reinterpretation greatly alters the significance of the publication, as its central conclusion, that grasslands, and specifically C₄ grasslands, arose early in the Eocene in South America, is unsubstantiated. It is important to note however that while the application of our correction factors alters the possibility of major grassland ecosystems at this time, it is clear from the data of Zucol et al. (2010) that grasses existed as a component of the vegetation in South America during the Eocene, which agrees with many other recent publications (e.g., Edwards et al., 2010).

4.3 Further work

As suggested above, much work remains to be done in terms of quantifying bias for all common soil order types and developing appropriate correction factors for use in all future paleoenvironmental reconstructions from phytoliths. In addition, there is room for significant work focused on parsing out differences in production versus preservation biases via plant-specific phytolith analysis, and in examining potential time-averaging of phytolith signals within

soils via high-resolution depth profile analysis. Further analysis of a broader range of sampling sites should also be undertaken in an effort to determine whether environmental or climatic factors could explain any remaining variability in bias estimates.

In addition to this body of work, past paleoenvironmental interpretations based on phytolith records (as with case study above) should be reconsidered in an effort to shed light on the origins of grassland ecosystems and their role in the evolving landscape of the Cenozoic. Such work is essential to the use of phytoliths as a proxy for biome classifications, and may have significant implications for fields like geoarchaeology as well, providing a potential tool for tracking land use change either on human timescales for high-resolution modern studies, or on ecological timescales in deeper time.

5. CONCLUSIONS

The primary goal of this work was to highlight the extreme representational bias present in modern phytolith assemblages in an attempt to dissuade other workers from directly interpreting paleo-assemblages for the purpose of paleoenvironmental reconstructions. Based on our calculated correction factors (~62% for Alfisol/Spodosols, ~6% for Inceptisols, and a negligible correction for Mollisols) for soil order types, it is crucial that future work in this field include characterizations of paleopedology, including accurate paleosol taxonomy and placement of paleosol and phytolith data into both temporal (i.e., vertical stratigraphic) and spatial (i.e., horizontal facies variation) context. Not only should future work contain such factors, past work should be reevaluated to ensure accuracy with respect to how well phytolith assemblages reflect original plant biomass and ecosystem type. These steps are essential to the continued use of phytoliths as a proxy for past vegetation and as a basis for ecological reconstructions throughout the Phanerozoic.

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FIGURE CAPTIONS

Figure 1. Generalized extent of studied soil orders of the United States (modified from NRCS).

Black dots indicate field sites (n = 18).

Figure 2. Common phytolith morphotypes from modern soil samples: A. bilobate (Bi-6), B. square plate (Blo-1), C. saddle (Sa-1), D. smooth (Elo-1 and Elo-7) and spiny (Epi-11) elongate, E. conical (Co-2) and keeled (Kr-4) rondel, F. trichome (Tri-4), G. crenate (Ce-4), H. knobbed block (Blo-6) and tracheary body (Tra-1), and I. palisade mesophyll (Ho-1 and M-1). All images taken at 1000x magnification, and black bar is 10 μm long in each image. See Table S1 for morphotype/compound affiliations.

Figure 3. Percent of total assemblage for each phytolith compound variable and corresponding plant category from example sites MO-A, an Alfisol, and 95.1-C, a Mollisol. White bars indicate the percent of the total aboveground vegetation that falls into the associated plant/compound category, while black bars indicate the percent of the total soil phytolith assemblage for the same category (described in Table S1).

Figure 4. Mean % Forest Indicator phytoliths and % Forest Indicator biomass for assemblages of the studied Soil Orders. Space between data points represents average bias for that Soil Order. Horizontal black lines indicate average counting error for phytolith and biomass estimates.

Figure 5. Environmental variable vs. bias regressions: A. Physical and experimental variables including phytolith yield, elevation, and soil texture, B. Climatic variables including mean annual precipitation (MAP), mean annual temperature (MAT), and effective energy and mass

transfer (EEMT), and C. Soil Order type, the only significant variable explaining variance in bias estimates. Ellipses indicate distinct bias clusters with standard error (2σ) limits plotted from cluster mean.

Figure 6. Interpreted percent grass in Gran Barranca vegetation during the Eocene shown in black (Zucol et al., 2010), and reinterpretation using proposed correction factors from this work in gray. Left column includes ages and phytozones described in Zucol et al. (2010), and horizontal lines indicate sample horizons (paleosols). Vertical dashed line represents the accepted cutoff for savannah or grassland ecosystems (Anderson, 1999).

Table 1. Previous studies involving bias in phytolith records.

Table 2. Site localities and associated environmental and climatic data.

Supplementary data:

Figure S1. Moisture availability vs. abundance of morphotypes within GRASS-D compound group. Best fit logarithmic trendline indicates a very weak relationship between these variables ($R^2 = 0.22$).

Table S1. Vegetation estimates from % cover and % biomass, with associated bias estimates.

Table S2. Phytolith morphotype classifications with associated compound groups and total sample counts.

Figure 1.

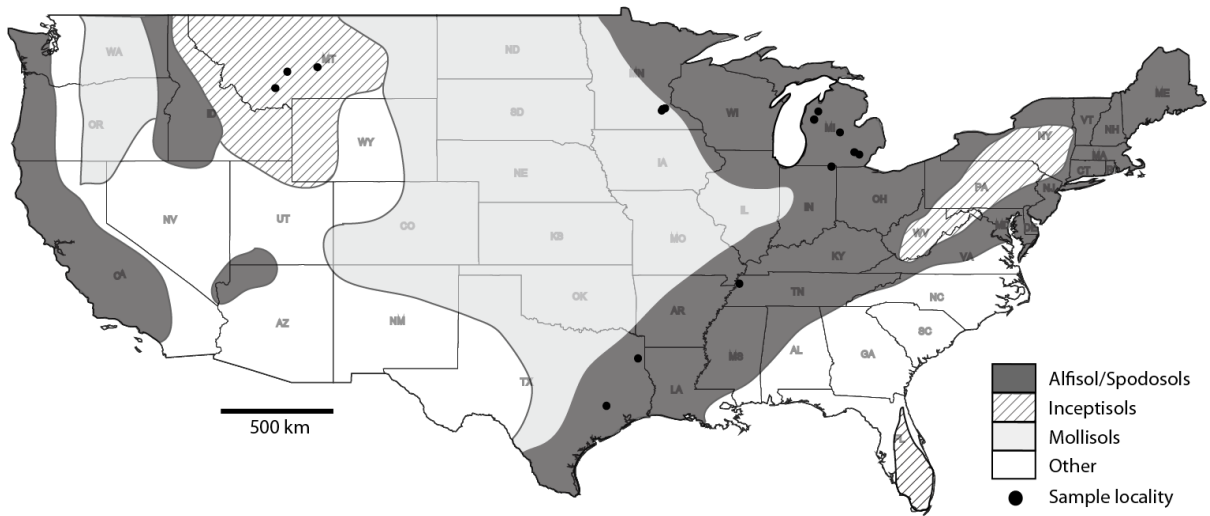


Figure 2.

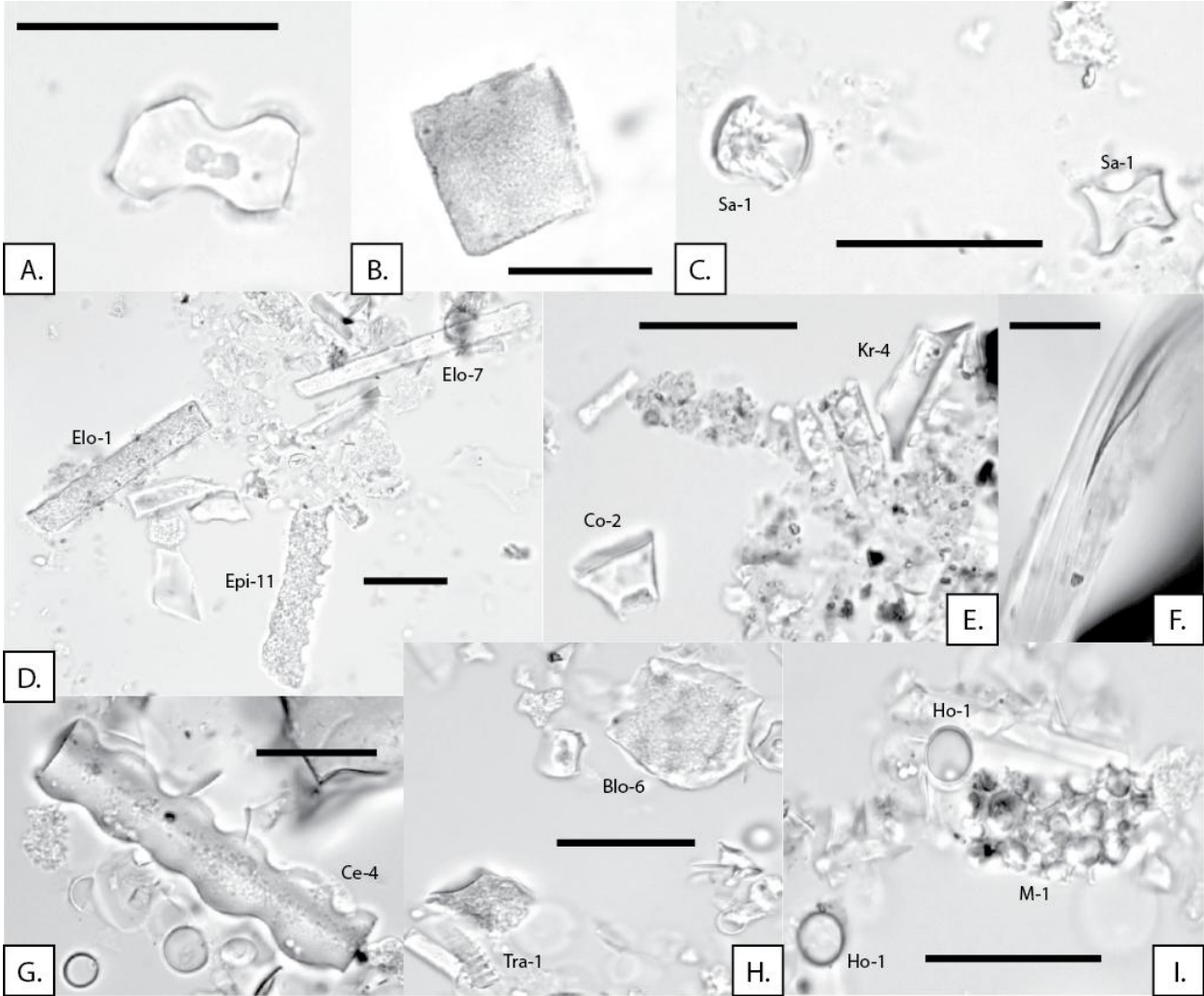


Figure 3.

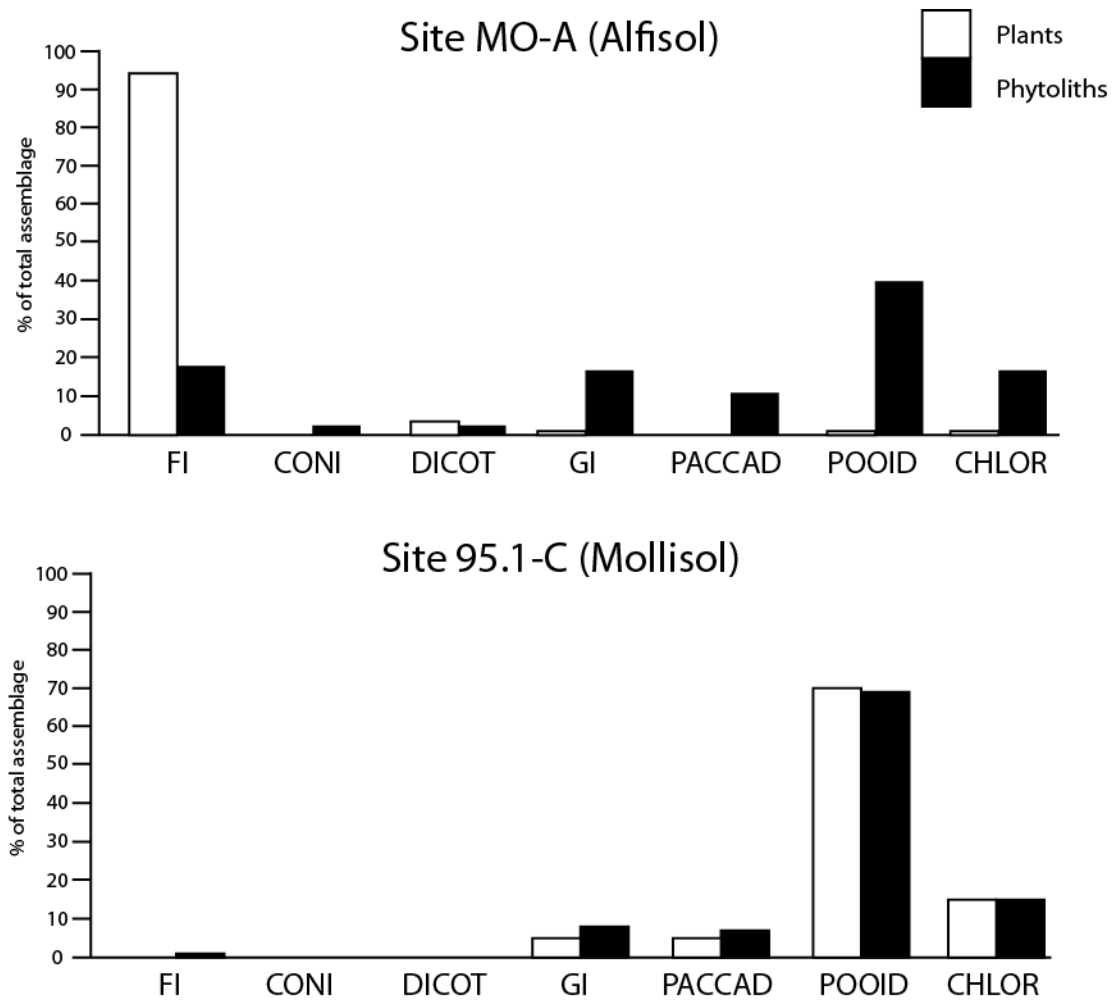


Figure 4.

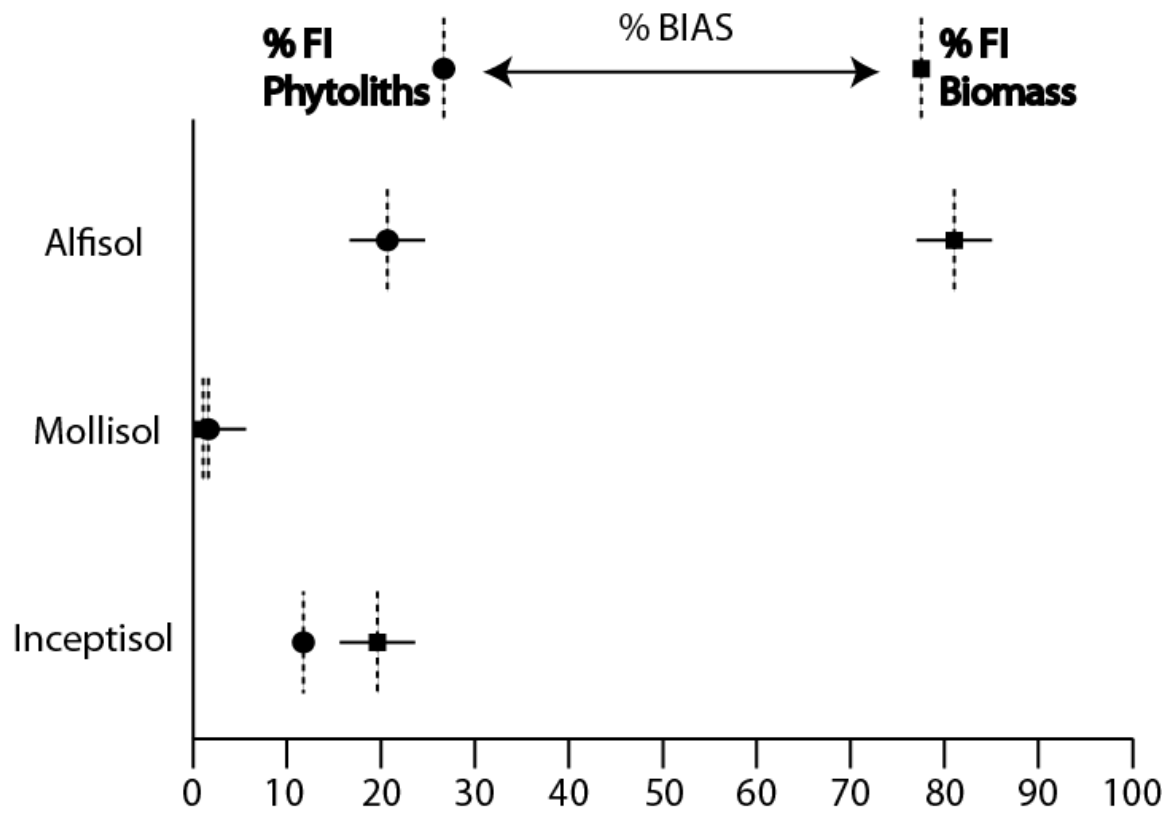


Figure 5.

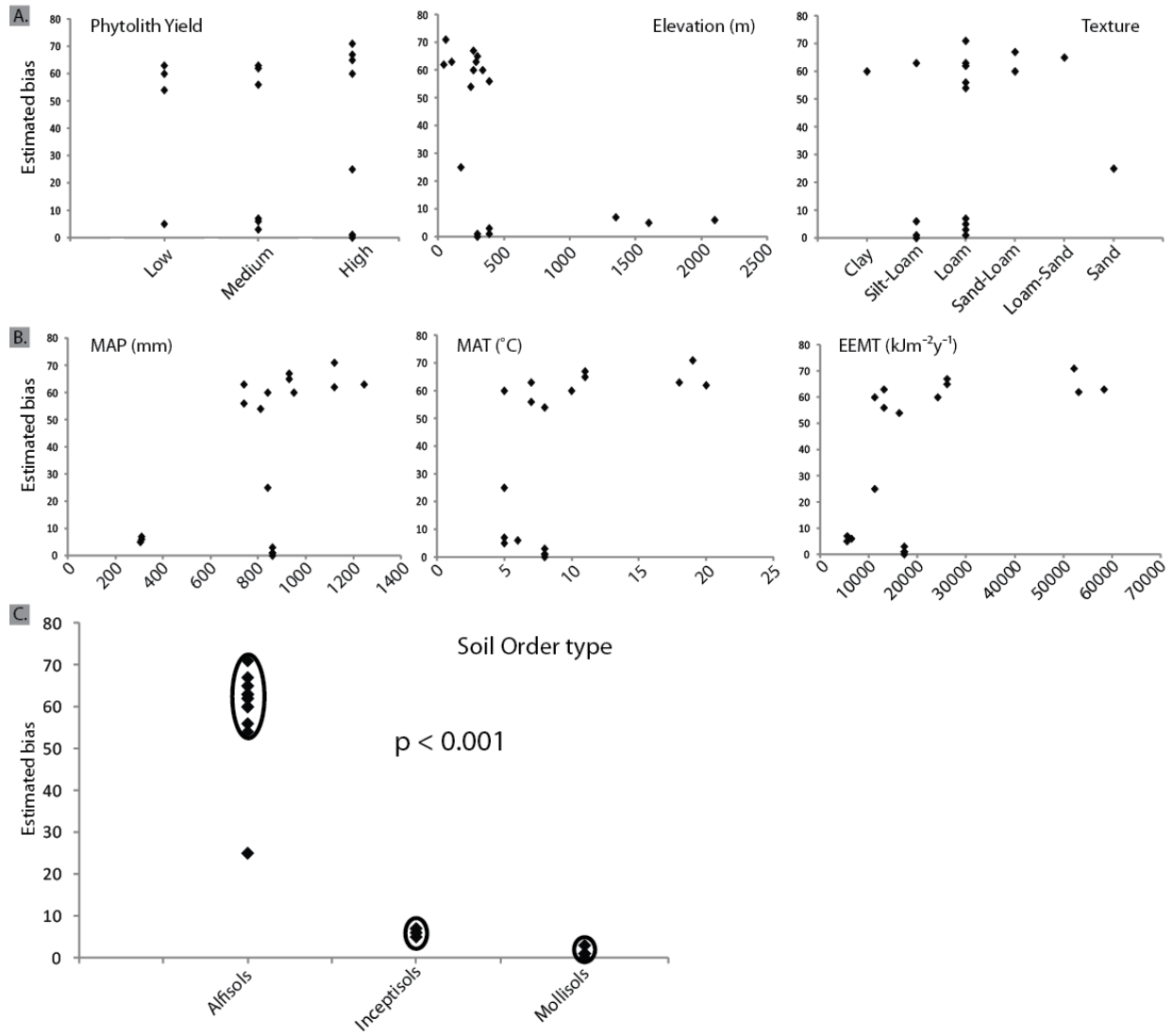


Figure 6.

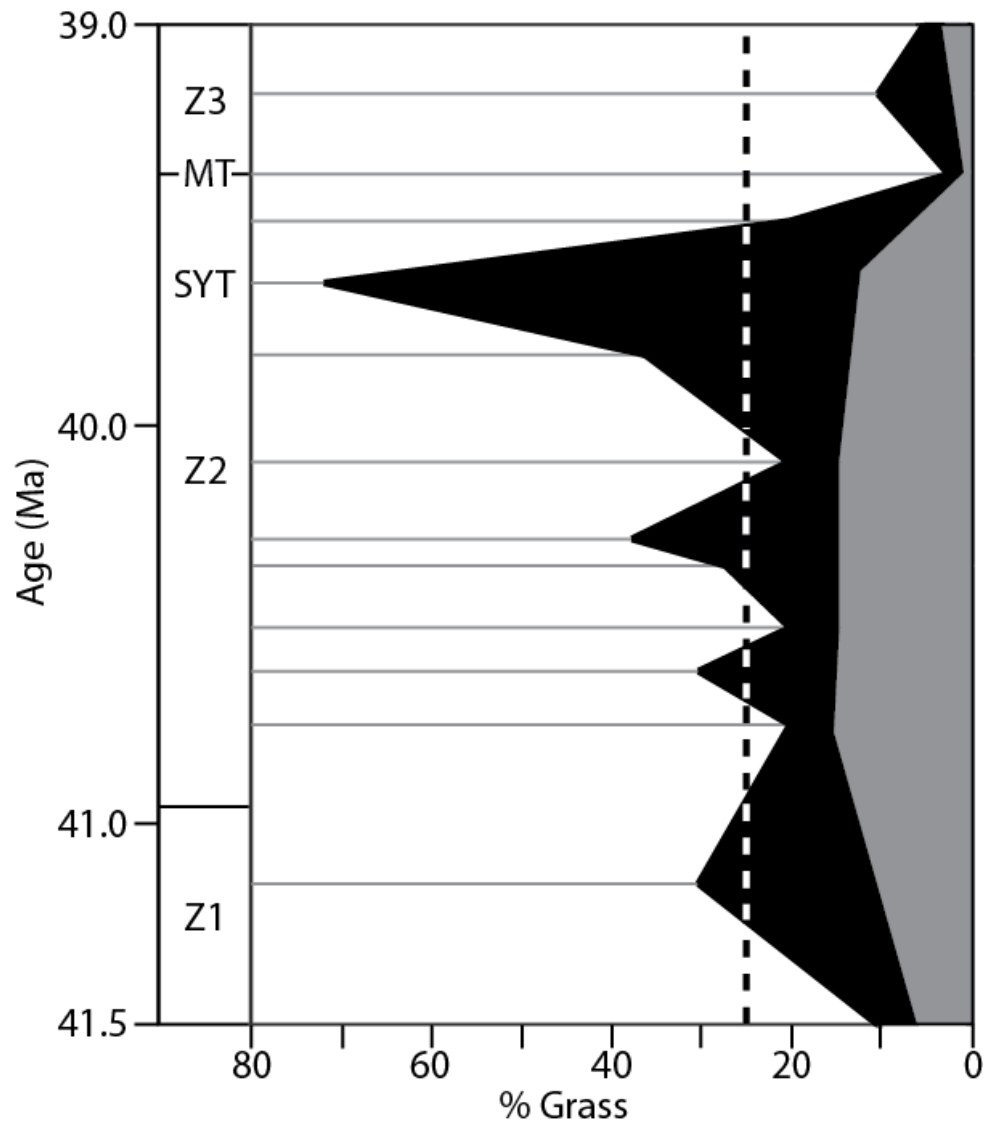


Table 1. Previous studies involving bias in phytolith records.

Study	Location	Ecosystem type	# of sites ^a	Bias analysis?	Assemblage results
Fredlund et al. (1994)	Central Plains, USA	Temperate grasslands	50	Yes	C ₄ grasses are underrepresented among grasses, phytolith assemblages are regional
Alexandre et al. (1997)	Senegal/Congo, AFR	Tropical forest, grasslands	2 (33)	Not addressed	Forest/grasslands are distinguishable, D/P function developed to compare them
Carnelli et al. (2001)	Valais Alps, SW	Alpine forest, heathlands	28	Yes (in plants)	Grasses are biased (1-12x more productive than other groups)
Kerns (2001)	Arizona, USA	Temperate forest	1 (27)	Yes	Forest Indicators are underrepresented (amount not quantified)
Blinnikov (2005)	Pacific NW, USA	Temperate forest, grasslands	58	Not addressed	Coniferous forest/grasslands are distinguishable
Bremond et al. (2005)	Mauritania/Senegal, AFR	Tropical grasslands	62	Yes	Grass phytoliths are representative of grassland biomass types
Bremond et al. (2005b)	Kandara, CM	Tropical forest, grasslands	26	Yes	Forest Indicators are underrepresented, bias relationship is predictable (LAI vs. D/P)
Borba-Roschel et al. (2006)	Minas Gerais, BR	Tropical grass/peatlands	1 (11)	Yes	Grass phytoliths are representative of grassland biomass types
Lu et al. (2006)	China	Tropical/temperate forest, grasslands	243	Not addressed	C ₃ /C ₄ phytoliths are distinguishable, assemblage composition is tied to climate
Barboni et al. (2007)	Central Africa	Tropical forest, grasslands	149	Yes	Forest/grasslands are distinguishable, Forest Indicators are underrepresented (by 20-45%)
Tsartsidou et al. (2007)	Greece	Mediterranean	62	Yes (in plants)	Grasses are biased (up to 20x more productive than other groups)
Bremond et al. (2008)	Kenya/Tanzania, AFR	Tropical forest, grasslands	3 (24)	Yes	C ₃ /C ₄ ratios are representative, forest/grassland ratios are nonrepresentative
Barboni et al. (2009)	East Africa	Tropical grasslands	184	Not addressed	C ₃ /C ₄ ratios are representative (qualitative)
Iriarte et al. (2009)	Uruguay	Subtropical forest, grasslands	9	Yes	Forest/grasslands are distinguishable, Forest Indicators are underrepresented (>50%)

^a Parenthetical numbers indicate the combined number of samples taken from sites.

Table 2. Site localities and associated environmental and climatic data.

Site name	Location	Soil order	USDA type name	MAT (°C)	MAP (mm)	EEMT (kJm ⁻² yr ⁻¹)	Elevation (m)	Phytolith yield ^a
MO	Ann Arbor, MI	Alfisol	Boyer loamy sand	11	930	26099	300	High
OH	Pinckney, MI	Alfisol	Fox sandy loam	11	930	26099	270	High
JP	Baldwin, MI	Spodosol	Gorvan-Houghton clay	5	840	11222	175	High
NH	Mesick, MI	Alfisol	Manistee sand	5	840	11222	340	High
OS-1	Northfield, MN	Alfisol	Ostrander loam	7	740	13123	290	Medium
OS-2	Dundas, MN	Alfisol	Ostrander loam	7	740	13123	390	Medium
C1-35	Waller, TX	Alfisol	Katy loam	20	1120	53162	45	Medium
C1.5-25	Marshall, TX	Alfisol	Bernaldo loam	19	1120	52206	60	High
C3-53	Dyersburg, TN	Alfisol	Memphis silty loam	18	1245	58383	105	Low
C6-31	Fremont, IN	Alfisol	Wawasee sandy loam	10	950	24193	270	Low
C7-43	Ithaca, MI	Alfisol	Capac loam	8	810	16265	250	Low
95-1	McKnight, MN	Mollisol	Blooming silty loam	8	860	17298	300	High
95-2	McKnight, MN	Mollisol	Blooming silty loam	8	860	17298	300	High
95-3	Northfield, MN	Mollisol	Hayden loam	8	860	17298	390	High
95-4	Northfield, MN	Mollisol	Hayden loam	8	860	17298	390	Medium
MS-1	Broadwater, MT	Inceptisol	Amesha loam	5	305	5531	1600	Low
MS-11	Madison, MT	Inceptisol	Neen silty loam	6	310	6441	2100	Medium
MS-16	Wheatland, MT	Inceptisol	Musselshell loam	5	310	5571	1350	Medium

^a Yield categories are qualitatively based on density of diagnostic phytoliths per counting slide.

Figure S1.

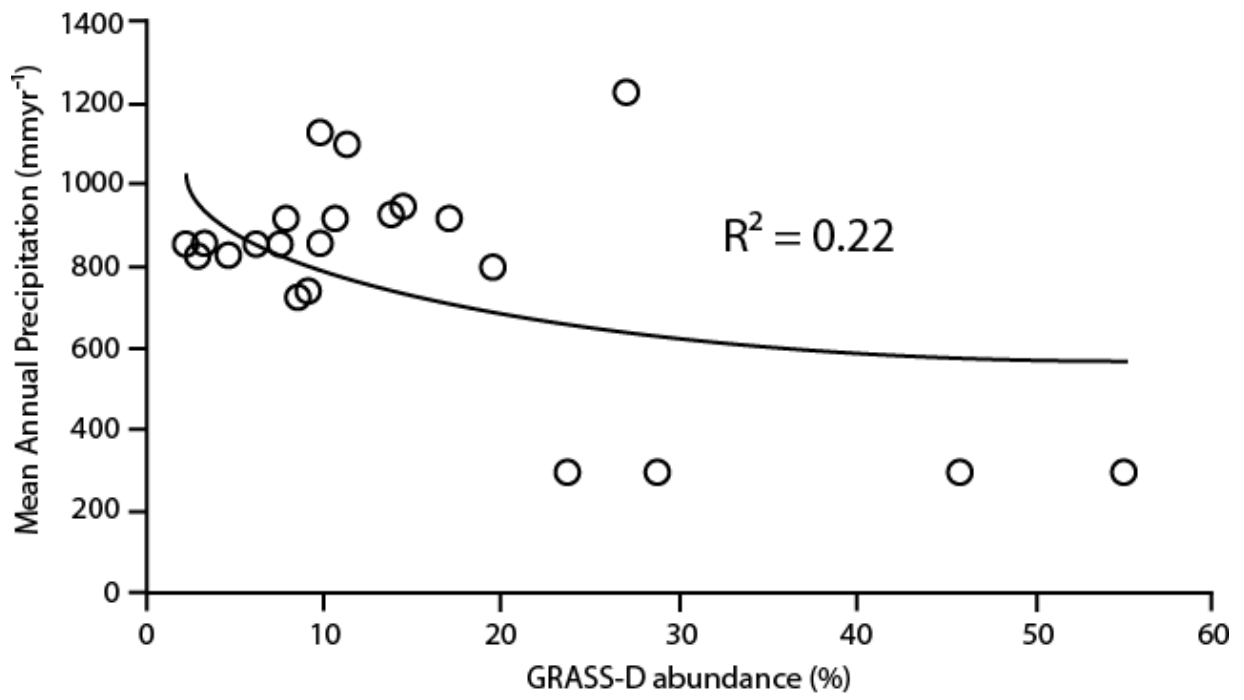


Table S1. Vegetation estimates from % cover and % biomass, with associated bias estimates.

	MO-A	MO-B	MO-C	OH	NH	OS-1	OS-2	JP	95.1-A	95.1-B	95.1-C	95.2	95.3	95.4	MS1-A	MS1-B	MS1-C	MS11	MS16	C1-35	C1.5-25	C3-53	C6-31	C7-43
Forest Cover (%)	100	100	100	100	100	45	40	100	0	0	0	0	0	0	20	20	20	5	10	90	100	100	80	85
Forest Biomass (%)	94	94	94	86	96	66	59	94	0	0	0	0	0	0	25	25	25	10	15	74	83	96	70	72
Grass Bias (%)	72	65	72	67	25	63	56	60	-3	0	-1	-1	-1	-3	*	5	6	6	7	62	71	63	60	54

* Indicates samples with insufficient diagnostic phytolith morphotypes for inclusion in statistical analyses.

Table S2. Phytolith morphotype classifications with associated compound groups and total sample counts.

Classification (Strömberg, 2003, 2005)	Compound	SITES																							
		MO-A	MO-B	MO-C	OH	NH	OS-1	OS-2	JP	95.1-A	95.1-B	95.1-C	95.2	95.3	95.4	MS1-A	MS1-B	MS1-C	MS11	MS16	C1-35	C1.5-25	C3-53	C6-31	C7-43
Knobby bodies (Kn-2)	CONI	4	1	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	3	8	1	0	0
Tracheary element (Tra-1)	FI-GEN	0	1	0	1	3	0	0	0	1	0	0	0	0	0	0	1	0	0	0	2	2	0	0	0
Palisade mesophyll (M-1/Ho-1 ^a)	FI-GEN	36	48	36	16	277	11	9	99	9	0	4	4	5	5	0	0	3	8	18	0	15	0	7	20
Knobbed blocks (Blo-6/Kn-1)	FI-GEN	0	14	17	0	0	0	0	0	0	0	0	0	0	0	0	32	19	0	0	21	0	0	11	0
Ovate (Cl-1)	DICOT-GEN	4	5	5	25	14	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	11
Tricome (Tri-4)	DICOT-GEN	0	0	0	0	0	0	0	0	0	1	0	0	0	6	0	12	17	0	0	4	3	68	3	10
Vertebral bodies (M-7)	GRASS-D	6	3	1	0	0	2	3	0	4	2	1	4	2	2	0	0	0	0	0	0	0	0	0	0
Spiny elongates (Epi-11)	GRASS-D	28	30	27	17	12	28	26	14	5	5	22	32	32	18	6	33	46	124	107	28	13	18	20	34
Bilobate (Bi-6/Bi-7)	PACCAD-GEN	11	14	22	12	12	29	22	27	17	25	25	13	13	24	0	0	0	0	5	13	28	2	7	0
Rondel (Co-2)	POOID-D	37	44	56	115	71	185	197	72	301	128	129	288	257	124	10	44	51	55	55	59	65	80	74	118
Crenate (Ce-2/Ce-4)	POOID-D	0	0	7	5	0	29	23	10	10	14	22	0	12	16	20	19	15	15	19	6	12	0	13	0
Keeled Rondel (Kr-4)	POOID-D	43	45	57	0	0	0	0	12	0	70	84	0	0	95	8	57	45	0	0	36	0	0	61	0
Saddle (Sa-1/Sa-2/Sa-5)	CHLOR	32	36	37	27	24	67	54	61	23	62	53	33	29	38	0	0	3	24	28	82	72	0	11	14
Bulliform (Blo-9/Blo-10)	GRASS-D	0	0	0	0	0	0	0	0	0	3	3	0	0	0	0	18	17	0	0	0	9	38	11	21
Elongates (Elo-1/Elo-7/Elo-11)	OTH	168	145	83	166	137	154	145	138	127	70	89	135	108	79	138	169	134	140	187	113	97	133	108	54
Rectangular (Blo-1/Blo-2/Blo-12)	OTH/ND	139	79	59	142	81	87	63	86	24	20	43	76	61	89	335	170	105	174	135	35	27	181	50	48
Diatoms/Sponge spicules	OTH	5	0	0	4	8	0	0	26	8	1	1	2	19	25	8	2	2	19	29	0	2	0	3	0
	TOTAL	513	465	407	530	639	592	542	548	529	401	476	587	538	521	525	556	460	559	583	402	353	521	379	330
	DIAGNOSTIC	201	241	265	218	413	351	334	298	370	310	343	374	350	328	44*	216	219	226	232	254	227	207	218	228

* Indicates samples with insufficient diagnostic phytolith morphotypes for inclusion in statistical analyses.

^a Indicates new morphotype category based on the work of Bozarth (1992).