ECOSCIENCE

8 (4) : 478-488 (2001)

Estimating forest-grassland dynamics using soil phytolith assemblages and $\delta^{13}C$ of soil organic matter!

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Abstract: Our objectives were to examine the relationship between contemporary vegetation and surface soil phytolith assemblages, and use phytoliths and $B^{13}C$ of soil organic matter (SOM) to explore forest-grassland vegetation dynamics. We established plots within three canopy types (open, old-growth, and dense young pine) with different grass species compositions in a *Pinus ponderosa* forest in northern Arizona and collected vegetation data and surface (0-2 cm) and subsurface (2-7 cm) mineral soil samples. Surface soil phytolith assemblages strongly reflected vegetation at the site scale (within several km^2), Local vegetation patterns (<50 m^2) associated with overstorycanopy types were weakly detected. Significantly fewer C_4 grass and ponderosa pine phytoliths were found in subsurface compared to surface soils. Surface $B^{13}C$ values showed no difference among canopy types. Subsurface $B^{13}C$ values were significantly (+0.83‰ more enriched in l3C than surface values. Phytolith assemblages and $B^{13}C$ of SOM reflect long-term accumulation of organic matter in soils and may not mirror contemporary vegetation for many reasons, including spatial shifts in species distribution and productivity. Considering all our phytolith and $B^{13}C$ evidence, we suggest that C_4 grasses were more widely distributed but less abundant, grasses were more spatially continuous, total grass productivity was greater, and species in the genus *Koeleria* and *Brotnus* were more common in the past.

Keywords: $\delta^{13}C$, C_3, C_4, 'orest understory, grasslands, non-metric multidimensional scaling, northern Arizona, opal, *Pinus ponderosa,* phytolith assemblages.

Résumé : Notre recherche à pour objectif d'examiner la relation entre la végétation contemporaine et les assemblages de phytolithes de la surface du sol et d'utiliser les phytolithes et le $\delta^{13}C$ contenus dans la matière organique du sol (MOS) afin d'explorer la dynamique de la végétation de forêt-prairie. Nous avons établi des placettes dans trois types de couvert (pinède ouvert, pinède à l'équilibre et pinède jeune) comprenant des assemblages différents d'espèces herbacées dans une forêt de *Pinus ponderosa* du Nord de l'Arizona et avons récolté des données de végétation ainsi que des échantillons de surface (0-2 cm), de sous-sol (2-7 cm) et de sol minéral. Les assemblages de phytolithes de surface reflétaient fortement la végétation à l'échelle du site (à l'intérieur de plusieurs km^2). Les patrons de végétation associés avec les types de couvert de l'étage forestier dominant ont été peu détectés. Significativement moins de phytolithes de graminées C_4 et de pin ponderosa ont été trouvés dans le sous-sol en comparaison avec les sols de surface. Les valeurs de $\delta^{13}C$ de surface ne montraient pas de différences entre les types de couvert. Les valeurs de $\delta^{13}C$ du sous-sol étaient significativement (+ 0,83 ‰) plus enrichies en ^{13}C que les valeurs de surface. Les assemblages de phytolithes et le $\delta^{13}C$ de la MOS reflètent l'accumulation à long terme de la matière organique dans les sols et peuvent ne pas refléter la végétation contemporaine pour plusieurs raisons, dont les changements dans la distribution des espèces et de la productivité. D'après nos données basées sur les phytolithes et le $\delta^{13}C$, les graminées C_4 étaient plus largement distribuées mais moins abondantes, les graminées étaient dispersées de façon plus continue, la productivité totale des graminées était plus élevée et les espèces des genres *Koeleria* et *Bromus* étaient plus communes dans le passé.

Mots-clés : $\delta^{13}C$, C_3, C_4, sous-étage forestier, prairie, graduation multidimentionnelle, Arizona, opale, *Pinus ponderosa*, assemblages de phytolithes.

Introduction

In the American Intermountain West, factors associated with Euro-American settlement such as fire exclusion and suppression, historical periods of over-grazing, and episodic climatic events have created dramatic changes in ponderosa pine, *Pinus ponderosa* P. & **C.** Lawson, forests (Cooper, 1960; Covington *et al.,* 1997; Covington & Moore, 1994a,b; Dieterich, 1980; Fule, Covington & Moore, 1997; Mast *et al.,* 1999; Savage, Brown & Feddema, 1996; Swetnam & Baisan, 1996; White, 1985). Forests have changed from open park-like stands of older trees to denser stands of young, small-diameter trees. Concurrent decreases in understory herbaceous plant production and shifts in species composition would most likely accompany increases in woody plant abundance (Cooper, 1960; Covington *et al., 1997;* Covington & Moore, 1994b; Moore & Deiter, 1992); however, quantitative studies investigating understory reference conditions are lacking. Due to fire suppression and grazing practices, undisturbed reference sites no longer exist so proxy research techniques must be used. Two methods that are potentially promising tools for understanding vegetation dynamics in cases like this are phytolith and stable carbon isotope analyses.

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Phytolith analysis is playing an increasingly important role in environmental reconstructions and interpretation of past vegetative communities (Blinnokov, 1999; Carbone, 1977; Fisher, Bourn & Fisher, 1995; Fredlund, 2001; Fredlund, Bousman & Boyd, 1998; Jiang & Piperno, 1998; Kealhofer & Penny, 1998; Kurmann, 1985; McClaran & Umlauf, 2000; Rovner, 1971). Phytoliths are particles of hydrated silica formed in the cells of living plants that can be diagnostic at various taxonomic levels (Piperno, 1988). Upon death and decay of plant material, phytoliths remain in the depositional environment and can be used to examine vegetation history and dynamics. Because of differential silica production in grasses and trees, many soils beneath grassland vegetation typically contain significantly more phytoliths by mass than soils beneath forest vegetation (Jones & Beavers, 1964; Norgen, 1973; Wilding & Drees, 1971; Witty & Knox, 1964). Examination of phytolith concentration in soils can be used to decipher changes in grassland and forest ecotones through time (Fisher, Jenkins & Fisher, 1987; Miles & Singleton, 1975) while phytolith morphology and assemblages (the suite of diagnostic shapes found in a substrate) can provide more detailed taxonomic information regarding vegetation change. However, many questions remain concerning how phytolith assemblages form and the relationship of assemblages to vegetation. Although modern analog studies have shown that soil phytolith assemblages reflect contemporary vegetation, validating use of this tool to reconstruct past vegetation (Bozarth, 1993; Fisher, Bourn & Fisher, 1995; Fredlund & Tieszen, 1994; Kurmann, 1985), it remains unclear at what spatial scale assemblages accurately reflect plant communities.

Many investigators assume that phytolith assemblages in soils are primarily the result of decay-in-place mechanisms (Dimbleby, 1978; Piperno, 1988), but Fredlund and Teiszen (1994) found that soil phytolith assemblages were not very sensitive to local variability in grassland species composition in the North American Great Plains. In their study, differences in species composition corresponding with temperature and moisture gradients across the Great Plains were detected, but local differences in species composition (differences associated with the location where the soil was removed) were not clearly reflected in the phytolith assemblages. These authors suggested that decay-in-place mechanisms are not solely responsible for assemblage formation, and assemblages reflect the vegetation found within several hundred meters of a collection location.

Stable isotope analysis of organic carbon (C) from soil organic matter (SOM) has emerged as an effective tool to describe the dynamics of vegetation communities having both C_3 and C_4 photosynthetic pathways (Ambrose & Sikes, 1991; Connin, Virginia & Chamberlain, 1997; Dzurec *et al.,* 1985; McPherson, Boutton & Midwood, 1993; Schwartz *et al.,* 1986). Plants with the C_3 photosynthetic pathway are substantially more depleted in ^{13}C ($B^{13}C$ values average *-27‰)* compared to C_4 plants ($B^{13}C$ values average *-12‰)* (Tieszen & Boutton, 1989). Since SOM is predominantly derived from plants, and fractionation during decomposition is small (Ehleringer, Buchmann & Flanagan, 2000), variations in $B^{13}C$ values of SOM can accurately reflect the relative abundance of C_3 and C_4 plant contributions (Balesdent, Girardin & Mariotti, 1993; Tieszen *et al., 1997).*

The goal of this study is to provide much needed data regarding phytolith assemblage formation, understory reference conditions, and vegetation dynamics in a ponderosa pine-bunchgrass community located near Flagstaff, Arizona, U.S.A. Our first objective was to determine the relationship between contemporary forest and grassland vegetation patterns and surface soil phytolith assemblages. A key question was whether surface phytolith assemblages can be used to decipher differences in vegetation at local (<50 m-) spatial scales. Our second objective was to compare surface and subsurface soil phytolith assemblages, phytolith concentration, and $B^{13}C$ of SOM in order to estimate forest-grassland vegetation dynamics.

Material and methods

STUDY AREA

The study site was located in northern Arizona, U.S.A., within the Fort Valley Experimental Forest, 10 km northwest of Flagstaff. This area was chosen because it has never been harvested (although hazard trees have been felled) and because it was proximal to an ongoing ecosystem restoration project (Covington *et al.,* 1997). The approximately 2 km² site consists of gently rolling topography (0-5% slopes) with an average elevation of 2250 m. Mean annual precipitation is 56.7 cm, with approximately half of that falling as snow in the winter and the other half as monsoonal rains in July through September (Schubert, 1974). Soils are mapped as a complex of fine, smectitic Typic Argiborolls and Mollie Eutroboralfs that developed on Tertiary basalt flows and cinders (Miller *et al.,* 1995). Mean annual air temperature is 7.5°C with an average of 94 frost-free growing days.

Present-day forest structure is characterized by small patches (0.02-0.29 ha) of larger old-growth ponderosa pine trees in clumps of three or more (White, 1985) interspersed with dense thickets of younger, smaller ponderosa pine trees, and relatively open canopy areas (<0.01 ha) with bunchgrasses and other herbaceous plants. Understory species composition is dominated by native bunchgrasses, which will be described in detail in the results section of this paper. Common forbs include *Carex* sp., *Cirsium wheeleri* (Gray) Petrak, *Solidago velutina* DC., and *Lotus wrightii* (Gray) Greene. The only understory shrub found was *Ceanothus fendleri* Gray.

FIELD METHODS

Fifteen 40-m² circular plots were established within the three forest canopy types found on the site (5 plots per canopy type): 1) old-growth pine; 2) dense young pine thicket; and 3) open canopy. Plot size was chosen to be slightly smaller than the smallest patches of vegetation (Kenkel *et al.,* 1989), which were the grassy areas. We stratified our plots using these canopy types because we observed that grass species composition differed among them, creating a natural test for sensitivity of phytolith assemblages to local variability, and we were also interested in understory-overstory vegetation dynamics. Open canopy plots were located within open canopy native grass areas, a minimum of 8 m from an old-growth tree or stump. A few postsettlement trees or seedlings were present on some of

these plots (0-4 stems), the main criterion being that the canopy was open. Dense young pine thicket plots (14-58 stems per plot) were located within stands of trees that germinated around 1919 (Mast *et al.,* 1999) and had dark bark and a stem diameter of < 15 cm at 1.37 m above-ground (dbh). Old-growth plots had three to five trees that were all at least 120 years old. These trees are easily distinguished by their relatively large size (dbh > 30 cm) and yellow bark. Because our goal was to minimize variability except for vegetation, plots were located within the same soil complex (Miller *et al.,* 1995) on relatively level terrain and away from rocky outcrops. Results from 18 soil pits in the same area revealed that A-horizon texture was consistently silt loam (unpub!. data). We attempted to evenly space out our plots, but some were clustered in our attempt to control for variables such as outcrops and topographic anomalies.

At each plot, trees over 1.37 m in height were tallied, and tree condition class was recorded. Any questionable tree in terms of age (old-growth or not) was cored. Forest floor (O horizon) depth was measured at the plot center and 2 m north, south, east, and west of the plot center. Aboveground understory plant biomass was determined by harvesting a 1.5-m x 0.5-m rectangular subplot located 25 cm from the plot center along a randomly chosen azimuth. All herbaceous and shrub plants were clipped at ground level, separated by species, placed in marked paper bags, dried at 70°C for 24 hours, and then weighed. The forest floor layer was then removed from the subplot, and a composite mineral soil sample (0-2 cm depth) was produced from ten systematically located soil cores. After removing the surface samples, a subsurface composite sample (2-7 cm depth) was collected in a similar manner using the same sample core locations.

The 0-2 cm interval was chosen to represent the modern soil surface (Fisher, Bourn & Fisher, 1995; Pearsall, 1986; Piperno, 1988). Fredlund and Tieszen (1994) used 0-5 cm as the modern surface, and we concluded that a shallower sampling depth should better reflect contemporary vegetation patterns. The subsurface interval of 2-7 em was selected to represent the pre-modern surface because we wanted to limit sampling to the A horizon, which can be as shallow as 7 cm in some places in the study area. Phytoliths are usually less abundant in other genetic horizons, and we wanted to minimize differences due to pedogenesis. Although plant roots have been found to contain phytoliths, discrete identifiable forms generally do not occur in roots; much of the silica found in roots is in the form of small nodular aggregates (Piperno, 1988).

LABORATORY METHODS

Soils were air-dried and visible organic matter removed prior to passing soil through a 2-mm sieve. Phytoliths were extracted using a modified heavy liquid flotation technique (Pearsall, 1986). Modifications included organic matter removal by dry oxidization in a muffle furnace (400°C) for eight hours or overnight (Kalisz & Stone, 1984; Rosen, 1993), isolation of the 5-250 um size class, and use of sodium poly tungstate (2.33 mg m^{-3}) as the heavy liquid. Initial scans of phytolith material revealed that some phytoliths were obscured by organic matter so extracts were subsequently digested with 30% H_2O_2.

PHYTOLITH CLASSIFICATION AND MICROSCOPE SCANNING METHODS

Phytolith forms used for this study are shown in Table I along with their locally representative taxa. This classification system was developed from local flora, and detailed descriptions, illustrations, and nomenclature are provided in Kerns (2001). Poaceae phytoliths include short-cell forms from leaf epidermis material. Only short-cells were considered because they are fairly equal in size and silicification; therefore, they should be equivalently resistant to post-depositional degradation. The only non-grass phytolith form characterized and described in this study is the spiny body diagnostic for ponderosa pine (Kerns, 2001).

Vials containing dry soil phytolith extract were randomized and mounted in Canada balsam with no reference to sample location. To view phytoliths three-dimensionally, a 200-225 total cell count was made at 400x using a Standard 20 Zeiss biological microscope before the mounting medium had dried. Only phytolith forms identifiable as one of the eight diagnostic types shown in Table I were counted. All phytolith assemblage results are reported as percentages, rather than absolute values. Powers-Jones and Padmore (1993) suggest that relative values appear to have fewer problems than total counts and are a reliable basis for multivariate statistical techniques.

^{14}C AND $\delta^{13}C$ ANALYSIS OF SOIL ORGANIC MATTER

Soil organic matter was dated using radiocarbon analysis in order to provide a relative measure of chronological control. Because of the high cost of ^{14}C analysis, subsets of two samples per canopy type were analyzed for ^{14}C. Surface and subsurface samples from two randomly chosen plots in each canopy type were submitted to the University of Arizona Laboratory of Isotope Geochemistry for TAMS (tandem accelerator mass spectrometer) radiocarbon dating using a Quantulus 1220 and a Rackbeta 1217. Samples were simultaneously analyzed for $^{13}C/^{12}C$ as required for ^{14}C determination. Sample preparation included root and particulate organic matter removal by hand and flotation in water, carbonate removal using 2M HCl, and humic acid extraction *(i.e.,* solubilization in 0.5 NaOH followed by precipitation with concentrated HCl; Dzurec *et al., 1985).*

TABLE I. The phytolith forms used in this study and their dominant locally representative subfamilies and genera (Kerns, 2001). Representative taxa are listed from most to least common for that phytolith form.

Phytolith Form	Subfamily or tribe	Representative taxa	Photosynthetic pathway
Pinus ponderosa			
spiny body	Pinaceae	*Pinus ponderosa*	C_3
Rondel	Pooideae	*Festuca, Poa,* all Pooideae	C_3
Pyramid	Pooideae	All	C_3
Stipeae pyramid	Stipeae Tribe	*Piptochaetium, Stipa*	C_3
Crenate	Pooideae	*Bromus, Koeleria, Elymus, Stipa, Poa*	C_3
Panicoid lobate	Panicoideae	*Schizachyrium, Panicum*	C_4
Simple bilobate	Varies	*Aristida,* all Chlorideae, Stipeae Tribe	C_4 or C_3
Saddle	Chlorideae	All	C_4

Dating material less than 200 ± 50 years old can be problematic because the sample will overlap the modem background $^{14}C/^{12}C$ ratio, which has been affected by industrialization (after 1650 AD) and atmospheric atomic bomb tests (starting in the 1940s AD) (Gillespie, 1984). When the measured ratio of the sample is close to or overlaps the modern background ratio, results are reported as percent modern. One hundred percent modem is the ^{14}C level in the atmosphere in AD 1950 after correction for industrial effects. Because of continual input of fresh C, SOM radiocarbon dates should always be interpreted as minimal ages; the actual age of phytoliths in the soil could be older. Wang, Amundson and Trumbore (1996) state that for the same soil type, measured ^{14}C ages of soil organic matter can provide some indication of relative age. All radiocarbon results in this study are reported as percent modem, with error terms reported as one standard deviation of measured precision. Stable carbon isotope results are expressed in standard $B^{13}C$ notation:

$$\delta^{13}C‰ = [(R_{sample} / R_{standard})^{-1}] \times 10^3$$

where Rsampleis the $^{13}C/^{12}C$ molar ratio of the sample and $R_{standard}$ is the $^{13}C/^{12}C$ ratio of the standard (Pee Dee Belemnite).

DATA ANALYSIS

Data were analyzed at two spatial scales: (1) pooled data from the site using all fifteen plots; and (2) data by canopy type, representing local (<50 m²) variability in vegetation and phytolith assemblages. Because our diagnostic phytolith classification system was limited to grasses and ponderosa pine, only grass species composition was examined. Analysis of variance was used to test for differences among the three canopy types in total above-ground grass biomass, selected phytolith forms (ponderosa pine-type and C_4-type phytoliths), and phytolith concentration. If the differences were significant ($p < 0.10$), pairwise tests were conducted using Tukeys procedure for multiple comparisons. Analyses of differences between surface and subsurface data were conducted using paired t-tests with a Bonferroni correction factor (ex determined by the total number of statistical tests). All phytolith counts were transformed (square-root) to correct for over-represented forms and strengthen under-represented forms (Fredlund & Tieszen, 1994; Overpeck, Webb & Prentice, 1985). Statistical analyses were conducted using SYSTAT 8.0 (SPSS Inc., Chicago). Only selected phytolith forms were used for univariate tests at the canopy scale because testing all eight phytolith morpho types among the three different canopy types and two depths would have resulted in 48 statistical tests. Therefore, trends in species composition and assemblages were examined with the ordination technique described below.

Non-metric multidimensional scaling (NMDS) has been shown to be one of the most effective methods available for the ordination of species and other taxonomic composition data (Kenkel & Orloci, 1986; Kruskall, 1964; Minchin, 1987). Unlike Principal Component or Detrended Correspondence Analyses, NMDS will not produce curvilinear distortions of underlying gradients (Minchin, 1987). Moreover, NMDS is based on fewer assumptions, and these assumptions are more consistent with observed patterns in vegetation (Prentice, 1980; Minchin, 1987). The most important property of an NMDS ordination is that the relative distances among the pairs of points and ordination axes are arbitrary and have no significant meaning. Examination of plots of stress, a normalized measure of badness of fit, *versus* the number of dimensions (1-4) provides a guide to the minimum number of dimensions required (Kruskall, 1964). For the ordinations presented in this paper, two axes appeared to adequately represent the data sets.

We also used vector fitting (Kantivilas & Minchin, 1989; Faith & Norris, 1989) to examine the relationship between different variables associated with forest structure and contemporary vegetation and phytolith assemblages. Vector fitting is a type of multiple linear regression that finds the direction across the ordination along which the sample scores are maximally correlated with a given variable. Rank-order categorical variables can be used and are similar to the use of dummy variables. Significance was tested using a random permutation procedure (1000 permutations, $p < 0.10$), and vector lengths plotted on ordinations were scaled proportionally to r^2 values. Four aspects of forest structure were tested as explanatory variables: live tree density, forest floor depth, and the rank-order categorical variables stand age (open = young; dense young pine = intermediate; and old-growth = old) and light (open = high light; old-growth = intermediate; and dense young pine = low light). Selection of these aspects of forest structure was based on our observation of factors that might control or characterize species composition at the site.

Ordinations and vector fitting were performed using the program DECODA 3.0 (ANUTECH, Pty. Ltd., Canberra). Species biomass and raw phytolith count data were transformed (square-root), standardized to species maxima, and transformed again into a Bray-Curtis dissimilarity matrix (Peter R. Minchin, pers. comm., 1999; Faith, Minchin & Belbin, 1987). Faith, Minchin and Belbin (1987) showed that this combination of standardization and use of the Bray-Curtis dissimilarity matrix was very effective for ordination of community data.

Results

CONTEMPORARY VEGETATION PATTERNS

Grasses made up 70% of the total above-ground understory herbaceous and shrub biomass measured on the 15 plots (data not shown). Table II lists all grass species and C_3 and C_4 relative abundance at the site scale. C_3 grasses dominated the species composition. Open canopy plots had significantly more grass biomass compared to the dense pine canopy plots and old-growth plots (Figure 1). C_4 species dominated open canopy plots, while no C_4 species were found on old-growth or dense young pine plots (Figure 1). Among C_3 species, *Elymus elymoides* (Raf.) Swezey was the most common and was found within all canopy types, while *Festuca arizonic a* Vasey and *Poa fendleriana*

TABLE II. Grass species relative abundance and photosynthetic pathway at the site scale (N = 13; two subplots had no grass species present). Data are mean percentages (± SE).

	Percent of biomass	Photosynthetic pathway
SPECIES		
Elymus elymoides	54.2 ± 11.1	C_3
Poa fendleriana	21.2 ± 10.8	C_3
Muhlenbergia montana	13.0 ± 6.8	C_4
Blepharoneuron tricholepis	6.4 ± 3.9	C_4
Festuca arizonica	4.7 ± 4.7	C_3
Muhlenbergia minutissima	0.4 ± 0.3	C_4
TOTAL		
C_3	80.2 ± 8.6	
C_4	19.8 ± 8.6	

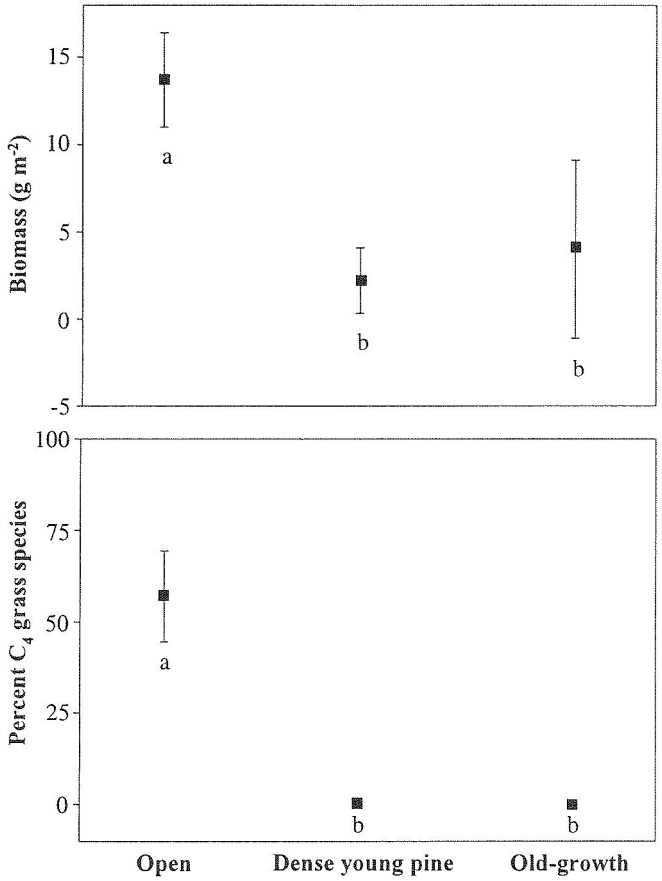


FIGURE 1. Mean (± SE) grass biomass and C_4 percentage for the three canopy types (N = 5). Different lowercase letters denote statistical significance among canopy types (p < 0.10; Tukey HSD). For old-growth and dense young pine plots, C_4 species were absent.

(Steud.) Vasey were found only in old-growth plots. Ordination results illustrate the correspondence between grass species composition, overstory canopy type and forest structure variables (Figure 2). Grass species composition was significantly correlated with three forest structure variables: stand age (r^2 = 0.74), forest floor thickness (r^2 = 0.71), and light (r^2 = 0.54).

SURFACE AND SUBSURFACE SOIL PHYTOLITH ASSEMBLAGES

If surface phytolith assemblages are sensitive to local variability in vegetation, we would expect that an ordination

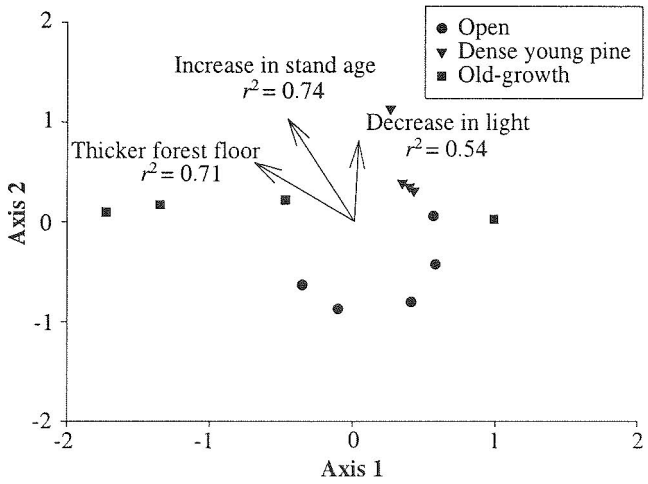


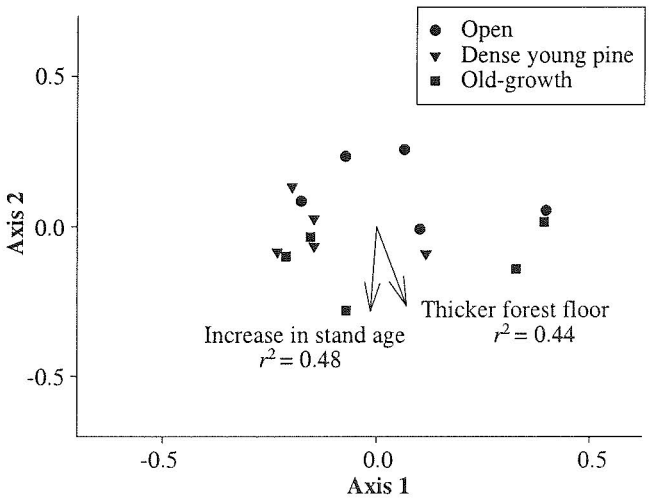
FIGURE 2. NMDS ordination of contemporary grassland vegetation, associated canopy types, and significant (p < 0.10) forest structure variables. Vector direction reflects maximum correlation between sample scores and the variable. Vector lengths are scaled proportional to r^2 values. Only 13 points are included because two subplots (one old-growth and one dense young pine had no grass species).

of the surface assemblages would show groupings among canopy types and significant forest structure variables that were similar to those shown for the contemporary vegetation. We would also expect open canopy plots to have a higher percentage of C_4-type phytoliths, while old-growth and young dense pine plots would have a higher percentage of the ponderosa pine spiny body.

The ordination of surface phytolith assemblages shows that these assemblages weakly correspond to present-day forest structure and grassland vegetation (Figure 3). Two out of the three significantly correlated variables for the contemporary vegetation ordination were also significantly correlated with the surface phytolith assemblage ordination (stand age, r^2 = 0.48; and forest floor thickness, r^2 = 0.44). However, no significant difference was detected for the percentage of C_4-type phytoliths or ponderosa-pine-type phytoliths among the three different canopy types (Table III). Mean percentages for all morphotypes for each canopy type and depth are shown in Appendix 1.

For surface assemblages, the highest percentage of the spiny body form, diagnostic for ponderosa pine, was found on old-growth plots (Table III). Although these plots do not have the greatest number of trees, they have much thicker forest floor accumulations of needle litter. To examine this relationship, we regressed the percentage of spiny body types against forest floor thickness, but this relationship was not significant. Interestingly, for the open canopy plots only, forest floor thickness was highly correlated with spiny body percentages (r^2 = 0.96). That is, for the open canopy type, plots with the thickest forest floor had the highest percentage of ponderosa-pine-type phytoliths.

At the site scale, it is apparent that the surface phytolith assemblage strongly reflected the overall species composition of the site (Table IV). The most abundant phytolith forms were the ponderosa pine spiny body and the C_3-type ronde!. Saddles (C_4 Chloridoideae type) made up only 8.8% of the assemblage. Very few panicoid lobate phytoliths were recorded, consistent with the rarity of grasses in the



scores and the variable. Vector lengths are scaled proportional to r^2 values.

TABLE III. Comparison of phytolith assemblage results by canopy type from surface and subsurface samples ($N = 5$). Data are mean percentages (± SE). No statistical differences ($p > 0.10$; Tukey HSD) were detected among canopy types or between depths ($p > 0.10$; Bonferroni, $p < 0.03$).

		Canopy type	
Phytolith form	Open	Dense young pine	Old-growth
Pinus ponderosa SPINY BODY			
SURFACE	48.6 ± 6.2	49.9 ± 5.8	59.9 ± 2.0
Subsurface	49.0 ± 5.8	41.6 ± 5.7	45.3 ± 3.6
C_4-TYPE PHYTOLITHS			
SURFACE	10.7 ± 1.4	11.5 ± 1.7	8.3 ± 1.3
Subsurface	10.6 ± 1.5	9.9 ± 1.9	9.2 ± 1.4

TABLE IV. Mean (± SE) subsurface and surface phytolith percentages at the site scale ($N = 15$). Asterisks denote statistical significance between depths ($p < 0.10$; Bonferroni, $p < 0.01$). n.s. = no significance.

Phytolith form	Surface	Subsurface	Significance
Pinus ponderosa spiny body	52.8 ± 3.0	45.3 ± 2.8	*
Rondel	26.8 ± 2.2	28.5 ± 2.3	*
Crenate	8.2 ± 1.0	11.4 ± 1.0	*
Pyramid	1.9 ± 0.3	4.6 ± 1.6	n.s
Saddle	8.8 ± 0.8	8.3 ± 0.8	*
Simple bilobate	1.2 ± 0.2	1.2 ± 0.3	n.s
Panacoid type	1.0 ± 0.1	0.3 ± 0.2	n.s
Stipeae pyramid	0.2 ± 0.1	0.3 ± 0.1	n.s

Panicoideae subfamily in the study area; likewise, very few Stipeae pyramid forms were found.

As expected, ordination results for subsurface soil phytolith assemblages revealed no correspondence with present-day forest structure and grassland vegetation (Figure 4). Unlike the surface phytolith assemblages, none of the forest structure variables significant for the contemporary vegetation ordination were significant for the subsurface assemblage ordination. No significant difference was detected for C_4-type phytoliths or ponderosa pine-type phytoliths among the three different canopy types (Table III).

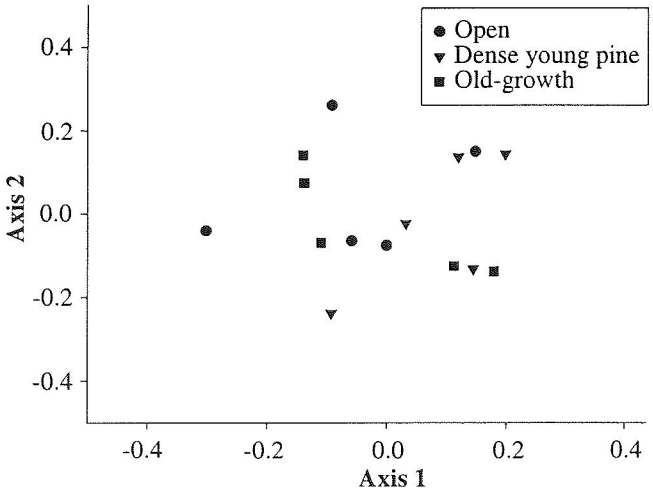


FIGURE 4. NMDS ordination of subsurface soil phytolith assemblages and associated canopy types. No significant ($p > 0.10$) forest structure variables were detected.

Comparison of surface and subsurface assemblages showed no significant differences for ponderosa pine spiny bodies or for C_4-type phytolith forms within any of the canopy types (Table III). At the site scale, significantly fewer saddles and ponderosa pine spiny bodies were found in the subsurface compared to the surface. Significantly more rondels and crenates were found in the subsurface compared to the surface (Table IV). No differences in mass recovery of phytolith material based on canopy type or depth were detected (Table V).

^{14}C AND $\delta^{13}C$ OF SOIL ORGANIC MATTER

Results from radiocarbon analysis of subsamples are shown in Table VI. Modern surface values all fall within a fairly close range, and subsurface ^{14}C values were significantly older than surface values when all plots were analyzed together ($p < 0.10$: Tukey HSD). These dates represent minimum average values of the humic acid fraction of soil organic matter. Therefore, our data support the assumption that the subsurface layer is older than the surface layer.

Despite the significant differences in C_3 and C_4 vegetation among different overstory canopy types, no significant differences were found for either surface or subsurface SOM $\delta^{13}C$ values among canopy types (Table VII). Open canopy plots, with an average of 40% C_4 grass carbon inputs (based on the two subplot subsamples), had values similar to those of the dense pine and old-growth canopy types, which have nearly pure C_3 carbon inputs. Mean surface SOM $B^{13}C$ values reflect dominantly C_3 input into this ecosystem (about 80%, assuming that all C_3 plants present on the plots have a $B^{13}C$ value of *-27‰* and all C_4 plants have a value of *-12‰)*. Subsurface $B^{13}C$ values were slightly (+ *0.83‰)* but significantly less negative than surface values when all plots were analyzed together ($N = 6$; $p < 0.10$; Tukey HSD).

Discussion

Surface phytolith assemblages strongly reflected the ponderosa pine and C_3 grass vegetation of the site. Very

TABLE V. Mean (± SE) phytolith concentration (g of silica • kg of oven dry soil^{-1}) for surface and subsurface soils for each canopy type (N = 5). No significant differences ($p > 0.10$; Tukey HSD) were found among canopy types or between depths ($p > 0.10$; Bonferroni, $p < 0.03$).

	Canopy type		
	Open	Dense young pine	Old-growth
Surface	0.9 ± 0.2	0.9 ± 0.2	1.4 ± 0.2
Subsurface	0.9 ± < 0.1	1.2 ± 0.1	1.3 ± 0.2

TABLE VI. Percent modern of ^{14}C of soil humic acid from different canopy types and depths. One hundred percent modern is the ^{14}C level in the atmosphere in 1950 AD after correction for industrial effects. Data are individual values from 2 plots in each canopy type, and the error associated with measurement precision.

	Canopy type		
	Open	Dense young pine	Old-growth
Surface	102.8 ± 0.5	103.2 ± 0.8	100.8 ± 1.0
Subsurface	99.1 ± 0.6	97.4 ± 0.8	97.6 ± 0.7
Surface	106.7 ± 0.8	98.7 ± 0.6	102.4 ± 0.7
Subsurface	97.0 ± 0.6	98.7 ± 0.6	100.3 ± 0.6

TABLE VII. Mean (± SE) $\delta^{13}C$ (‰) values of soil humic acid from different canopy types and depths (subsampled, N = 2). Measurement precision was ± 0.1‰. No significant differences were detected among canopy types ($p > 0.10$; Tukey HSD). Subsurface $\delta^{13}C$ values were significantly (+0.83‰) less negative than surface values when all plots were analyzed together (N = 6; $p < 0.10$; Tukey HSD).

	Canopy type		
	Open	Dense young pine	Old-growth
Surface	-23.9 ± 0.2	-23.9 ± 0.7	-24.4 ± 0.2
Subsurface	-22.0 ± 0.2	-23.5 ± 0.3	-23.5 ± 0.4

few phytolith forms were found in surface assemblages that did not have a modern analog at the site although some species that produce these phytolith forms (Stipeae pyramid,panicoid lobates, simple bilobates) occur within several kilometers of the area. Local vegetation patterns (<50 m^2) associated with overstory canopy types were weakly detected. The surface phytolith assemblage ordination revealed some correspondence to contemporary vegetation; however, even on open canopy plots dominated by C_4 species and having few trees, the percentage of C_4 and ponderosa pine type phytoliths was not statistically different compared to forested plots where C_4 species were absent.

Our results are consistent with those of Fredlund and Tieszen (1994), where local differences in species composition associated with the location where the soil core was removed were detectable but only weakly reflected in surface assemblages. However, Fisher, Bourn and Fisher (1995) found that above-ground vegetation and phytolith assemblages collected at 0-2 cm depth from Capitol Reef National Park, Utah, were highly correlated. Inconsistencies among these studies could be due to differing methodologies and numerous factors that influence phytolith assemblages. Fredlund and Tieszen (1994) suggest that processes such as dispersal and inheritance are important for phytolith assemblage formation. In grasslands, fire and herbivory can be important mechanisms for dispersal of phytoliths (Fredlund & Tieszen, 1994). Inheritance refers to the incorporation of phytoliths into the soil over several hundred years and is a function of plant community stability, decomposition rates, soil accumulation and erosion rates, geomorphic stability, sampling depth, and phytolith resistance to degradation. Thus, assemblages should be viewed as a long-term average of vegetation composition, rather than an instantaneous snapshot of modern vegetation. Although we sampled at a shallower depth than Fredlund and Tieszen (1994) in order to capture a modern analog, organic matter decomposition rates may be slower at our semi-arid site.

Factors such as fire, herbivory, and erosion might be responsible for the lack of local sensitivity in our phytolith assemblages. It is also likely that understory species are not spatially stable through time. Forests of the U.S. Intermountain West have experienced dramatic recent changes in land-use history and episodic climatic events (Covington *et al.,* 1997; Dieterich, 1980; Fule, Covington & Moore, 1997; Mast *et al.,* 1999; Savage, Brown & Feddema, 1996; White, 1985). We hypothesize that in the past C_4 grasses were more widely distributed. This would explain why no difference was detected in the surface and subsurface soils for C_4-type phytoliths and $\delta^{13}C$ values among canopy types. Our data indicate that C_4 grasses were not more abundant in the past as we found significantly fewer C_4-type phytoliths in subsurface compared to surface soils. It is possible that C_4-type phytoliths degrade more quickly in soils or that C_4 grasses do not produce as many phytoliths as C_3 grasses. However, Piperno (1988) suggested that short-cell production of C_4 grasses can be greater than C_3 grasses.

If C_4 grasses were relatively less abundant compared to C_3 grasses in the past, there are several mechanisms that could explain a recent increase in C_4 grasses. Higher minimum summer temperatures, lower soil moisture, and higher solar radiation favor C_4 plants (Ehleringer, 1978; Teeri & Stowe, 1976). Increased temperature associated with global warming could be a factor although some research suggests C_3 species are favored by increased CO_2 concentrations (Cole & Monger, 1994; Polley et al., 1993). Over-grazing that took place in the study area between 1876 and 1920 (Covington et al., 1997) and preferential selection of C_3 grasses over C_4 by herbivores (Akin & Burdock, 1977; Caswell et al., 1973) might be an important consideration. Pearson (1942) found that on plots dominated by *Festuca arizonica* Vasey, a C_3 grass, both grazing and experimental clipping resulted in the increased abundance of *Muhlenbergia montana* (Nutt.) A. S. Hitchc, and *Blepharoneuron tricholepis* (Torr.) Nash, C_4 grasses. Using phytolith analysis, Fisher, Bourn and Fisher (1995) found a similar historical (past 200 years) increase in C_4 grasses at Capitol Reef National Park, Utah, and hypothesized that grazing was responsible for the shift in species composition. Additionally, Kelly *et al.* (1991) mention that selective reduction of C_3 plants by grazing may explain discrepancies in $\delta^{13}C$ SOM values and standing biomass.

Subsurface $\delta^{13}C$ values were slightly (+ 0.83‰) but significantly higher than surface values. This result could be due to (1) increased production of C_4 grasses relative to C_3

grasses in the past or (2) an increase in total grass productivity relative to ponderosa pine productivity in the past. Because our phytolith data contradict the first hypothesis mentioned, we favor the second hypothesis. Even if C_4 grasses were less abundant relative to C_3 grasses in past, an overall increase in grass productivity relative to pine tree productivity would still increase $B^{13}C$ SOM values. This explanation is also consistent with the result that significantly fewer ponderosa pine spiny bodies were found in subsurface compared to surface soils. Although differential degradation might be a factor, Blinnokov (1999) found ponderosa pine phytoliths from ca 80 000 years ago in the Palouse loess from Columbia Basin grasslands. In our study, phytolith concentration was also similar among canopy types. Because grasses produce dramatically more silica compared to trees, this result could indicate that grasses were more spatially continuous in the past, lending additional support to the idea that past grass productivity was greater.

The $B^{13}C$ subsurface enrichment could also be unrelated to vegetation change. A common observation in C_3 ecosystems is a progressive enrichment in $B^{13}C$ SOM values with depth in the soil profile (Nadelhoffer & Fry, 1988; Ehleringer, Buchmann & Flanagan, 2000). Although this enrichment is generally thought to be related to decomposition, the mechanistic basis for this is controversial (Ehleringer, Buchmann & Flanagan, 2000).

Interestingly, no difference was found among canopy types for the ponderosa pine phytolith type. This could be due to the ubiquitous nature of ponderosa pine litter. Although open canopy plots had no or only a few trees present, measurable ponderosa pine litter was recorded on all of these plots (data not shown). Furthermore, only in open canopy plots was the recovery of ponderosa pine spiny bodies highly correlated with forest floor thickness. This result suggests that at high rates of ponderosa pine litter inputs the rate of above-ground accumulation of forest floor materials becomes uncoupled from the rate at which phytoliths are incorporated into the surface mineral soil.

One other important implication regarding vegetation dynamics emerged from our study. At the site scale, significantly more crenate phytolith forms were found in subsurface soils compared to surface. These phytoliths are diagnostic for *Koeleria macrantha* (Ledeb.) Schult. and native species in the genus *Bromus (i.e., Bromus ciliatus* L.) (Blinnokov, 1999; Kerns, in 2001). Although these species are presently uncommon in the study area, our results indicate that they were more abundant in the past. *Koeleria macrantha* and *Bromus ciliatus* are considered excellent quality forage (Allred, 1993), and over-grazing may be responsible for the decrease of these species in the study area. However, other factors may also be important, such as changes in fire frequency (Fule, Covington & Moore, 1997) or buildup of detritus that develops in the absence of fire (Knapp & Seastadt, 1986).

Although we note several caveats associated with both phytolith and stable carbon analysis, our study demonstrates that (1) phytolith assemblages strongly reflected vegetation at the site scale, and local patterns (<50 m^2) were only weakly detected; (2) C_4 grasses were likely more widely distributed but less abundant in the past; (3) grasses were probably more spatially continuous, and total grass productivity was greater in the past; and (4) species in the genus *Koeleria* and *Bromus* were probably more common in the past. Our study provides an important contribution to understanding phytolith assemblage formation, but additional research on the utility of coupling phytolith and isotopic approaches is needed to fully develop this tool for assessing vegetation change.

Acknowledgements

This research was supported by state funds provided by the School of Forestry, Northern Arizona University, the United States Department of the Interior Bureau of Land Management, Arizona State Office, and several Northern Arizona Graduate College Organized Research Grants. We are especially thankful to Lauren Labate for field and laboratory assistance. Deborah Pearsall, University of Missouri at Columbia, provided much needed expertise regarding phytolith morphology. Mike Wagner, Northern Arizona University, provided access to his laboratory and use of a standard Zeiss microscope. Very helpful reviews by Michael Wimberly, Misha Blinnokov, Jeff Behan, Daniel Binkley, and two anonymous referees were greatly appreciated.

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APPENDIX I. Mean (± SE) percentages of all phytolith morphotypes by canopy type ($N = 5$).

Phytolith form	Canopy type		
	Open	Dense young pine	Old-growth
SURFACE (0-2 CM)			
Rondel	28.6 ± 4.5	27.9 ± 5.1	24.0 ± 1.0
Pyramid	1.7 ± 0.4	2.6 ± 0.6	1.3 ± 0.3
Stipeae pyramid	0.2 ± 0.3	0.1 ± 0.1	0.2 ± 0.3
Crenate	10.2 ± 1.6	7.9 ± 1.6	6.4 ± 1.7
Panicoid lobate	0.4 ± 0.2	0	0.1 ± 0.1
Simple bilobate	1.4 ± 0.5	1.7 ± 0.3	0.6 ± 0.4
Saddle	8.9 ± 1.0	9.8 ± 1.9	7.6 ± 1.2
Spiny body	48.6 ± 6.2	49.9 ± 5.8	59.9 ± 2.0
SUBSURFACE (2-7 CM)			
Rondel	26.5 ± 4.1	33.0 ± 3.5	25.9 ± 4.4
Pyramid	1.9 ± 0.5	3.84 ± 0.9	8.0 ± 4.5
Stipeae pyramid	0.4 ± 0.3	0	0.5 ± 0.3
Crenates	11.7 ± 2.04	11.56 ± 2	11.1 ± 1.3
Panicoid lobate	0.4 ± 0.3	1.0 ± 0.6	0
Simple bilobate	2.0 ± 0.5	1.0 ± 0.6	0.8 ± 0.3
Saddle	8.1 ± 1.2	8.4 ± 1.9	8.5 ± 1.4
Spiny body	49.0 ± 5.8	41.6 ± 5.7	45.3 ± 3.6