Using Fire-Altered Phytoliths to Reconstruct the Indigenous Fire Regime at McCabe Canyon

Abstract

We tested three approaches based on using the identification of individual burned soil phytoliths (weathering-resistant microscopic particles of amorphous silica formed in plants and deposited in the soil) as fire proxies to reconstruct the prehistoric fire regime at McCabe Canyon in Pinnacles National Park. Each of the approaches was found to have serious problems. The first approach, identifying visually darkened phytoliths, was easy when performed with freshly burned leaf material, but highly problematic when confronted with the multitude of colors and opacities of weathered phytoliths extracted from a typical grassland soil. The second approach, using changes in the refractive index caused by heating of phytoliths, did not produce substantial changes within in the temperature range typical of grassland fires, and was prone to rehydration effects in the soil environment. The third approach, using Raman spectroscopy to identify changes caused by heating to the carbon occluded in phytoliths, was not able to identify heated phytoliths, but was only able to distinguish phytoliths that were visually darkened when subjected to an open flame. Each approach was rejected as inadequate; we were unable to use phytoliths to reconstruct the prehistoric fire regime at McCabe Canyon. Additional research quantifying the effects of weathering on soil phytolith coloration and opacity may make the first approach viable. High soil phytolith content in most areas of Pinnacles NP that are currently California Annual Grassland suggests that these areas were native grass-dominated grasslands for centuries prior to European settlement, making them prime candidates for restoration of their prehistoric plant communities.

I. Background and Purpose

1. Introduction

Despite considerable qualitative information regarding Indian fire regimes in Californian grasslands and shrublands, primarily derived from ethnographic interviews of tribal elders, obtaining quantitative data to test hypotheses of fire frequency and intensity has been problematic because a lack of trees and lakes in these environments renders typical approaches such as fire-scar dendrochronology and charcoal analysis of lake sediments unfeasible (Stephens et al. 2007). Recent advances in the paleoecological technique of phytolith analysis have provided the framework for promising, but to this point incomplete and untested, new approaches to reconstructing rangeland fire regimes. We have further developed and tested these approaches in an attempt to apply it to our study area to produce the first phytolith-based estimate of a fire regime in California.

Opal phytoliths are microscopic particles of amorphous silica that are formed when soluble silica taken up by plants from the soil solidifies within cells (often taking the shape and incorporating the nucleus and other contents of the cell) and between cell walls (Piperno 2006). Many phytoliths are highly resistant to decomposition, often persisting for hundreds or thousands of years (Wilding 1967). Recent research suggests that several changes occur to phytoliths when they are heated or exposed to fire:

1) Phytoliths (normally transparent) change color, becoming dark or opaque (Parr 2006)
2) The refractive index (RI) increases; the ratio of phytoliths with RI >1.44 to those <1.44 is >1 for burned phytolith samples (Elbaum et al. 2003)
3) The nature of carbon occluded in phytoliths, measured by spectra from Raman spectroscopy (focusing a laser beam on individual phytoliths), permanently changes as a function of maximum temperature exposure (Pironon et al. 2001).

Although these approaches have not been adequately tested and calibrated for field use, each has been used to identify individual burned phytoliths. The first approach, using changes in the proportion of darkened phytoliths to total phytoliths in the soil profile, has been used by several researchers to infer changes in fire frequency over time in grassland environments (Kealhofer 1996; Boyd 2002; Gu et al. 2008; Morris et al. 2010).

2. Objectives

A. Develop and test the phytolith coloration, refractive index, and Raman spectroscopic approaches for identifying burned phytoliths.

B. Use one or more of these approaches to reconstruct past fire frequency and intensity at the McCabe Canyon site.

C. Sample soil phytoliths at current grassland sites within Pinnacles National Park to determine the extent of long-term grasslands and if long-term, estimate their prehistoric fire regimes.

II. Study Description and Location

1. Study Areas

The investigation was centered in McCabe Canyon, a recently acquired area within Pinnacles National Park, focusing on two areas within the canyon with sizable patches of two species likely favored by Native Americans and maintained by regular burning: deergrass (with saddle-shaped short cells found only in *Muhlenbergia* among common native California grasses) and whiteroot sedge (with conical cells only found in Cyperaceae). Intensive sampling of plants and soil was undertaken at the deergrass patch both prior to and following the prescribed burn performed December 2011. Phytoliths previously extracted from samples in both archaeological and natural contexts at Quiroste Valley were also examined for evidence of burning (Evett and Cuthrell 2013).

2. Study Methods

To examine the effects of heating and burning on phytoliths of deergrass and whiteroot sedge, we conducted both laboratory and field experiments. Laboratory work involved heating vegetative samples of each species in a muffle furnace to temperatures at 100°C intervals from 100-600°C. Additional samples were burned using an open flame in a beaker under the laboratory hood. Heated and burned samples were further processed in household bleach for two days to remove residual organic matter and then rinsed several times in distilled water.

To examine evidence of burning through changes in phytolith color or opacity, a drop of phytolith solution from each sample was placed on a microscope slide and examined under a microscope at 400x magnification. The proportion of phytoliths showing evidence of burning was estimated by counting 300 phytoliths for each sample.

To investigate changes in the refractive index of phytoliths occurring as a result of heating, a portion of each phytolith extract was dried then re-suspended in Cargille Refractive Index Liquid with refractive index 1.44. A drop of each suspension was placed on a slide and examined under the microscope (Elbaum et al. 2003). By observing the nature of the Becke line (Elbaum et al. 2003; Wyche 2012), 200 saddle short cell phytoliths from each sample were counted and classified as either above or below 1.44 RI, and the percentage of phytoliths above 1.44 RI was calculated.

Raman spectrometry was performed using two instruments, a Renishaw RM1000 at the Spectral Imaging Facility at University of California, Davis, and a confocal Horiba LabRAM HR at the Analytical Chemistry Instrumentation Facility at University of California, Riverside. A drop of aqueous suspension of each phytolith sample was placed on a piece of aluminum foil taped to a slide (to avoid contamination with the spectrum of the glass slide, which is composed of amorphous silica similar to phytoliths), allowed to dry, and then placed under the microscope. Individual phytoliths were located on each slide and the laser beam focused within the phytolith at 1000x. Samples were examined using two laser light wavelengths, 523 nm and 784 nm. Raman spectra were obtained for dozens of phytoliths from each sample using exposure times ranging from 10 seconds to 2 minutes. Raman spectra of individual phytoliths were closely examined to identify predictable spectral patterns that manifest with increasing temperature.

The field portion of the study had several components. At the deergrass site in McCabe Canyon, soil samples and specimens of major plant species that occur in the area were collected prior to the prescribed burn in December 2011and phytoliths were extracted following established phytolith sampling procedures (Evett et al. 2006; Evett et al. 2007) to establish a baseline soil phytolith assemblage with interpretation based on a modern phytolith reference collection. Prior to the burn, aluminum tags painted with temperature-sensitive lacquers from 79-204°C (Tempilaq) were placed in NPS delineated sample plots in order to estimate the range of maximum temperatures occurring during the burn. Samples of ash were collected from each plot immediately following the burn. These were examined under the microscope at 400x before and after treatment with bleach to determine the proportion of visually darkened phytoliths resulting from the burn. To estimate the rate of incorporation of burned phytoliths into the soil after the occurrence of two rainy seasons following the burn, soil samples were collected from each plot late spring 2013 and phytoliths extracted.

To determine the extent of long-term native grasslands in Pinnacles National Park, soil samples were collected from sites currently dominated by California annual grassland. Phytoliths were extracted and the presence of long-term grassland determined using the 0.30% soil phytolith content threshold (Evett et al. 2013). In an attempt to estimate vegetation change over time, phytoliths were also extracted from undated chronologically stratified sedimentary layers from several cut bank sites located in streambeds.

Rather than assume the proportion of burned to total phytoliths in a soil phytolith assemblage produces a reasonably accurate quantitative estimate of long-term fire frequency, we attempted to test and calibrate this approach by extracting soil phytoliths from ponderosa pine-bunchgrass sites along a fire frequency gradient on NPS lands in the Rincon Mountains of southern Arizona. These sites were chosen because there are fire frequency maps constructed from well-documented historical and dendrochronological fire histories extending back several hundred years that document relatively stable fire frequency continuing through the present, a highly unusual situation given the introduction of fire suppression in most forests 100+ years ago (Farris et al. 2010). Sample sites were located within patches of grass in the forest (because the assumed long-term grass understory in the pine forest was expected to produce abundant burned phytoliths for analysis) along a gradient ranging from <10 yr fire return interval (with frequent fires that continue today) to >100 years since the last fire; 2 soil samples were collected at each site and soil phytoliths were extracted.

III. Results

Prescribed Burn

The prescribed burn of the deergrass site in McCabe Canyon was very patchy. While most of the site showed evidence of burning, there was a wide range of charring of vegetation and ash production (Figures 1 and 2). Precise measurement of maximum temperatures achieved during the prescribed burn was not successful. At all but one of the plots, all of the temperature-sensitive lacquers melted on the aluminum tags, indicating the prescribed burn was considerably hotter than expected. The range of

temperatures measured by the lacquer clearly needed to be much higher.

Figure 1. Photograph of the western portion of the deergrass plot two weeks after December 2011 prescribed burn. Note the patchiness of colors representing burn intensity.

However, based on deergrass leaf samples that were exposed to a range of temperatures in the muffle furnace in the laboratory, an indirect method of estimating fire temperature using the color of the burned leaves was devised. Leaves and stems heated to 300°C in the muffle furnace that maintained their structure but showed considerable, incomplete black charring. Samples heated to 400°C showed complete black charring with some breakdown of leaf structure. At 500°C, samples were reduced to fine black ash, converting to fine white ash at 600°C. Using these parameters, an ocular estimate of the percentage area of the burn within each temperature class was: 15% from 0-200°C, 20% from 200-400°C, 10% from 400-500°C, and 55% >500°C. Because almost all of the aluminum tags were placed within individual deergrass plants where post-fire ash indicated the fire temperature was >500°C, it is not surprising their narrow temperature range failed. Two tags showed evidence of melted aluminum. Since the melting point of aluminum is 660°C, maximum temperatures at the site during the burn likely approached 700°C, considerably hotter than maximum temperatures recorded in grassland fires in Kansas and Florida (Gibson et al. 1990). This may be due to the extensive thatch buildup in the deergrass plot that had likely not been burned for decades.

Figure 2. Post-burn close-up of one of the burned plots with aluminum tag that was painted with temperature-sensitive lacquers. Note the wide range in the nature and color of the remaining vegetation and ash. Laboratory experiments suggest this indicates there was a very wide range of temperatures experienced at a small scale.

Using Phytolith Coloration to Identify Burned Phytoliths

Deergrass and ponderosa pine leaf samples burned with an open flame in a beaker in the laboratory produced very high percentages of phytoliths that were either opaque black or transparent but dark gray or dark brown (Table 1; Figure 3).

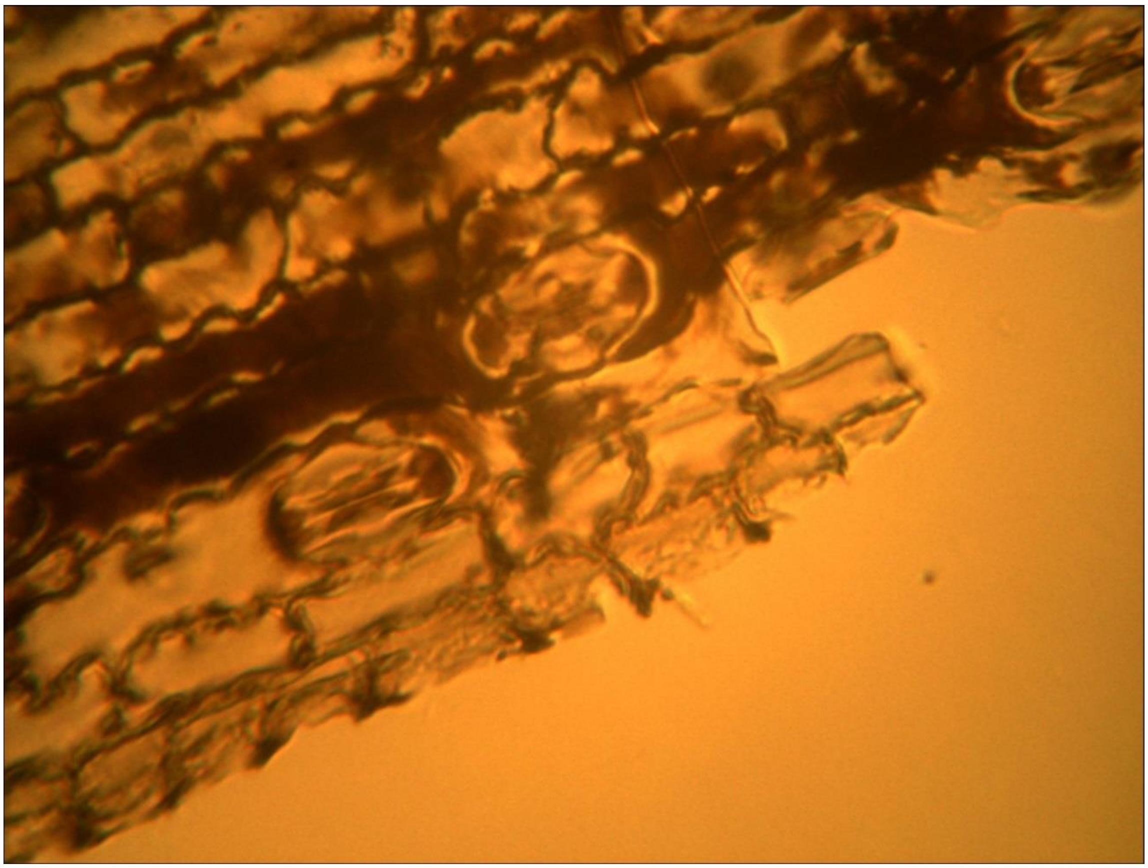


Figure 3. Deergrass leaf burned with an open flame in the laboratory at 400x magnification. Note the totally dark, opaque phytoliths upper right, partially colored phytoliths toward the middle, and normal uncolored phytoliths at the bottom.

A few phytoliths showed no color change as evidence of burning, even though they were in close proximity on the same leaf as phytoliths that changed color (Figure 3). A small percentage of phytoliths appeared very light gray or brown and were classified as ambiguous. However, deergrass leaf samples heated in a muffle furnace did not produce phytoliths with evidence of burning below 400°C, and only minimal percentages of burned or ambiguous phytoliths at higher temperatures (Table 1). Leaf samples heated above 300°C in the muffle furnace often smolder rather than ignite, with flames only appearing briefly; the low percentages of burned phytoliths observed in these samples probably reflect the brief flaming episodes. It appears that exposure to an open flame rather than heat is required to produce darkened phytoliths, contrary to the assertion of Pironon et al. (2001).

Table 1. Percentage of phytoliths identified as positively burned (black, dark gray, or dark brown), possibly burned (altered in some way to resemble burned), or ambiguous, from samples of various experimental manipulations and sampling sites.

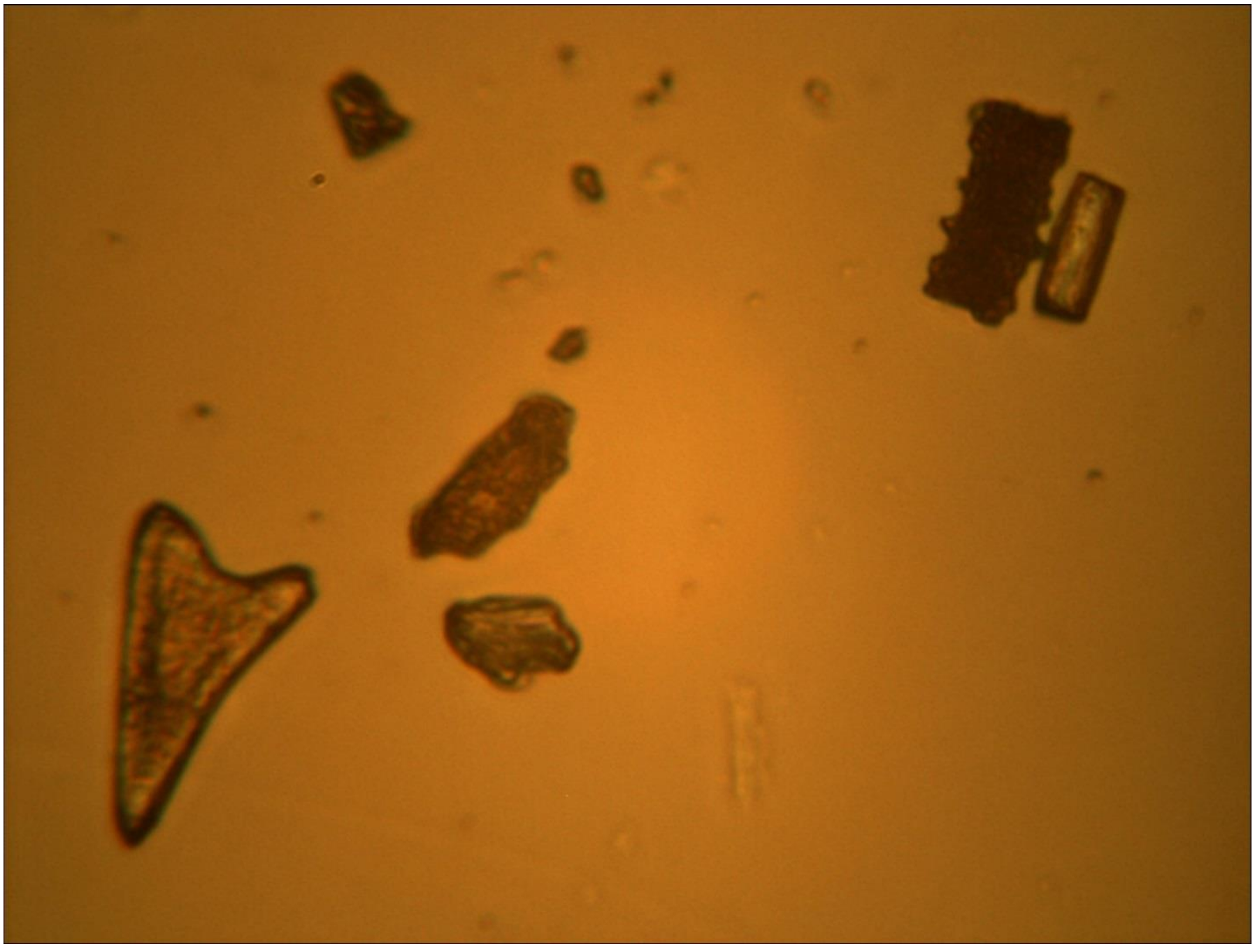
Experimental Manipulation or Sampling Site	Positively Burned (%)	Possibly Burned (%)	Ambiguous (%)
Ponderosa pine leaf lab burn	85	0	5
Deergrass leaf lab burn	92	0	4
Deergrass unheated 20°C	0	0	0
Deergrass muffle furnace 100°C	0	0	0
Deergrass muffle furnace 200°C	0	0	0
Deergrass muffle furnace 300°C	0	0	0
Deergrass muffle furnace 400°C	3	0	1
Deergrass muffle furnace 500°C	8	0	2
Deergrass muffle furnace 600°C	6	0	2
Prescribed burn ash samples (mean, 12 plots)	76	0	6
Pre-prescribed burn soil samples (mean, 12 plots)	2	22	18
Post-prescribed burn soil samples (mean, 12 plots)	7	26	14
Cut bank below prescribed burn site at 30cm depth	7	32	17
Pinnacle NP grassland soil samples (mean, 20 sites)	3	18	8
Pinnacle NP burned grassland near entrance	6	14	19
Quiroste Valley N25W103 020 Ash	11	46	24
Mascareignite from Reunion Island	6	29	22
Rincon Mountains FRI interval >60 yr (mean, 2 sites)	3	27	16
Rincon Mountains FRI interval 60 yr (mean, 2 sites)	5	14	7
Rincon Mountains FRI interval 30 yr (mean, 2 sites)	8	23	11
Rincon Mountains FRI interval 20 yr (mean, 2 sites)	4	19	13
Rincon Mountains FRI interval 15 yr (mean, 2 sites)	4	22	17
Rincon Mountains FRI interval <15 yr (mean, 3 sites)	8	28	17

Samples of ash taken from plots immediately after the burn have high percentages of colored phytoliths indicating burning (Table 1). Because burned phytoliths in the ash were identical to the phytoliths burned in the laboratory, there was little problem identifying color differences between burned and unburned phytoliths.

Burned phytolith percentages of soil samples taken from plots in the deergrass site before and after the prescribed burn were not substantially different, although as expected, a slightly higher percentage of burned phytoliths was observed post-burn. However, these values have little meaning; they are highly dependent on sample location because most of the recently burned phytoliths are still on the surface and not enough time has passed to incorporate them evenly along the surface or at depth into the soil.

Note that soil samples contain high percentages of phytoliths classified as “possibly burned”. These phytoliths show some alteration of color or opacity but they do not have the obvious black, gray, or brown darkening or reduced opacity seen in the laboratory burned or ash samples (Figure 4).

Figure 4. Examples of burned soil phytolith classification used in this study. The darkened serrate short cell (upper right) is classified “positively burned”. The smooth short cell (upper right) has a slight grayish brown color and is classified “ambiguous”. The trichome cell (lower left) is classified “possibly burned” because it is slightly opaque and brownish with a granular texture that does not match the color or texture of any of the laboratory-burned phytoliths but has some characteristics of burning that indicate it may be a burned phytolith subjected to weathering.



A big problem with identifying burned phytoliths in soil samples is that weathering of both burned and unburned phytoliths occurs over time and there has been no research examining how weathering changes the color and opacity of burned phytoliths compared to unburned phytoliths. Based on examination of phytoliths extracted from hundreds of soil samples from sites around the state (Evett and Bartolome 2013), the low percentages of unequivocally burned phytoliths seen in soil samples in this study are typical, as are the percentages of “possibly burned” phytoliths that show changes that may be due to burning or weathering or both.

Additional sites sampled at Pinnacles NP also showed few positively burned phytoliths (Table 1). A sample at 30 cm depth (age of this deposit is unknown) from a cut bank in an intermittent channel ~100 m below the deergrass prescribed burn site showed 66% of phytoliths that may have been burned; if all of these were indeed burned (a highly speculative and unlikely assertion), this would be evidence of frequent fire in the area. Phytoliths extracted from soil samples collected during the survey of current grassland areas in Pinnacles NP have lesser content of possibly burned or ambiguous phytoliths. A sample from a grassland area near the park entrance that was burned in 2009 showed a positively burned phytolith percentage comparable to the post-burn deergrass plot (Table 1). Even though a large number of darkened phytoliths are released into the soil following a burn event, compared to the vast pool of existing unburned soil phytoliths deposited in the soil by at least a century of grass production for grazing livestock with infrequent burning, the increase in the percentage of burned phytoliths expected in the soil as the result of one burn would be minimal.

Given the low positively burned phytolith percentages in the soils examined, to better understand the effects of weathering on burned phytoliths, samples were examined from two sites that would be expected to have high burned phytolith percentages and had also been subjected to weathering. The first site was an ash pit known to have very high phytolith content, deposited ~1000 yr ago at the Quiroste Valley archaeological excavation (Evett and Cuthrell 2013). While 81% of the phytoliths showed some alteration, only 11% were positively identified as burned (Table 1). The second site was from a soil on Reunion Island in the Indian Ocean composed of mascareignite, a mineral derived from burned bamboo phytoliths deposited during the past several thousand years (Meunier et al. 1999). This sample showed 57% of the phytoliths had some alteration, but only 6% were positively burned. These results suggest that most of the altered phytoliths observed in samples from Pinnacles NP could indeed be burned. However, the most powerful argument against altered, non-darkened phytoliths being burned comes from the Raman spectroscopy of individual phytoliths portion of this study, which found only phytoliths that were obviously darkened, not those altered in other ways, produced Raman peaks characteristic of burning (see Raman results section). The lack of knowledge of changes in burned vs. unburned phytoliths with weathering remains an obstacle to application of this approach to reconstructing fire regimes.

Phytoliths extracted from soil samples collected along a fire return interval (FRI) gradient from the Rincon Mountains in Arizona showed few that could be positively identified as burned (Table 1). All sites had total altered phytoliths between 30-50%, but there was no correlation between the FRI and any measure of altered phytoliths. This suggests that most of the non-positively identified altered phytoliths were not burned, but rather only weathered, since all sites were subjected to the same weathering regime. Another possibility is that sites would need to have an FRI more frequent than 10-15 years to make enough impact on the soil phytolith assemblage to raise the positively burned phytolith level above background noise.

In conclusion, using phytolith coloration to identify burned phytoliths in the soil appears to have some potential as an approach for reconstructing past fire regimes, but there are currently too many uncertainties, rendering the approach unreliable. While our burning experiments have demonstrated that a high percentage of phytoliths in leaves subjected to open flames change to a darker color, there are likely changes that occur to burned phytolith coloration following weathering in the soil that make them difficult to distinguish from weathered unburned phytoliths. Most previous research utilizing darkened phytoliths as an indicator of burning has been conducted in low-weathering conditions. For example, Boyd (2002) examined phytoliths buried in loess deposits, where most phytoliths were buried below the biological weathering zone near the surface before much weathering occurred. Likewise, Kealhofer (1996) examined phytoliths in lake sediments where weathering is greatly reduced. On the other hand, Morris et al. (2010) studying phytoliths in soils in Utah, made no mention of difficulty identifying burned phytoliths; however, burned phytolith percentages approaching 50% at sites with a recent fire but no long-term history of frequent fires are hard to understand unless some weathered unburned phytoliths were counted as burned. Regardless, even if all altered phytoliths do indeed represent burned phytoliths (contrary to the Raman results), our Rincon Mountains data suggests that unless fires are very frequent, the percentage of burned phytoliths in the soil phytolith assemblage does not change much.

Using Refractive Index to Identify Burned Phytoliths

There was little effect on the refractive index of phytolith samples heated for 10 minutes in a muffle furnace below 300°C (Figure 5). Above 300°C, the RI increased with increasing temperature. However, exposure to temperatures >480°C were required to exceed the 50% above 1.44 RI threshold used by Elbaum et al. (2003) to identify a phytolith sample that had been burned. Additionally, there were substantial decreases in RI resulting from rehydration of heated phytoliths when immersed in water for one week (Figure 5); phytolith samples heated to higher temperatures exhibited increased rehydration effects. Rehydrated samples did not cross the 50% threshold unless initially heated to nearly 600°C.

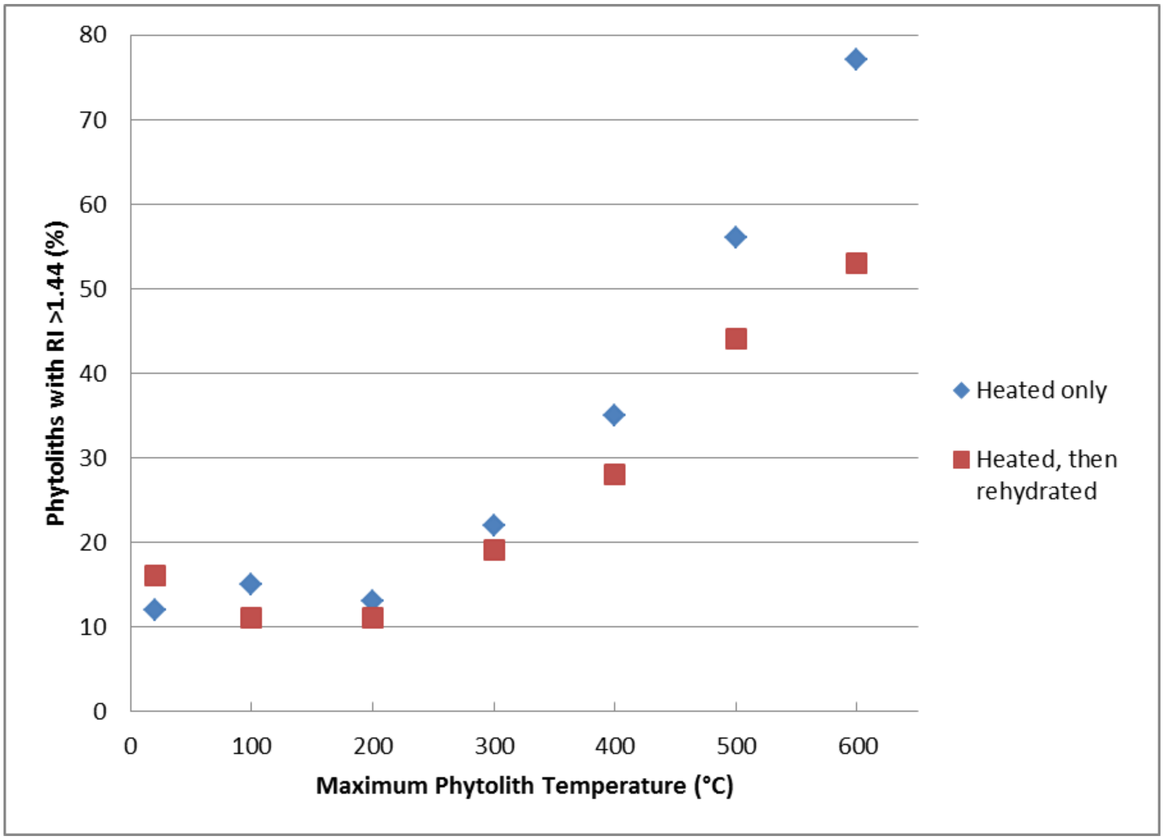


Figure 5. Percentage of saddle short cell phytoliths with refractive index (RI) above 1.44 after ashing deergrass leaf samples at various temperatures in a muffle furnace and after ashed samples were rehydrated for one week in distilled water.

The RI approach to identify burned phytoliths relies on the fact that heating of phytoliths that occurs through exposure to fire expels some of the water that is incorporated in the phytolith matrix during their formation. Because the RI of water at 20°C is 1.33 and the RI of pure silica is 1.54, the RI increases as more water is expelled at higher temperatures. However, our phytolith heating experiment shows there are several problems with this approach:

1) There are no obvious changes in phytolith RI that occur unless heated above 300°C. Many plants subjected to a typical grassland fire will not be heated above this temperature (Gibson et al. 1990) and will not record any changes in RI. This is particularly a problem for low intensity fires resulting from annual or biennial burning that may have characterized the Native American burning regime at Pinnacles NP.
2) Even if fire temperatures at a site routinely exceed 300°C, the percentage of phytoliths recorded as burned depends on fire intensity. Hotter fires produce a higher percentage of phytoliths with RI >1.44. Using the percentage of phytoliths with RI >1.44 as an index for fire frequency is not accurate because of the confounding effects of fire intensity.

3) Changes in RI likely occur due to rehydration when a burned phytolith is incorporated into the soil and exposed to soil moisture. This suggests the percentage of phytoliths above 1.44 RI in the soil phytolith assemblage as an index of fire frequency is confounded with the soil moisture regime.
4) The water content of phytoliths generally decreases with age (Piperno 2006), which means a phytolith that has persisted in the soil for hundreds of years will have a higher RI regardless of whether it was exposed to fire. Because a soil phytolith assemblage is composed of phytoliths along a continuum of ages and the age of an individual phytolith is unknown, this variable is also confounding.

The RI approach to detect burned phytoliths was developed to determine whether plants at an archaeological site were burned in a hearth, where temperatures usually exceed 1000°C (Elbaum et al. 2003). For this situation, the problems outlined above are not as significant because all phytoliths are heated to temperatures that increase the RI; fires in the hearth are hot enough to push nearly all phytoliths well above 1.44 RI; while rehydration likely pushes burned phytolith RIs lower, the majority remain above the 1.44 threshold; and the phytoliths in an archaeological feature such as a hearth are likely of similar age. However, our experiment has shown that trying to apply this approach to identify burned phytoliths from grassland fires is highly problematic and using this approach to estimate grassland fire frequency and/or intensity would likely be highly inaccurate. Other techniques (such as Fourier Transform Infrared Spectroscopy) that rely on phytolith water content to identify burned phytoliths have the same problems and are likely unsuitable as well.

Using Raman Spectroscopy to Identify Burned Phytoliths

Because the phytolith coloration and RI approaches to positively identify phytoliths exposed to fire were found not suitable for reconstructing grassland fire regimes, considerable time and effort (~250 hours in the Raman lab and spectra of ~1500 phytoliths examined) were expended testing the Raman spectroscopic approach. While the procedure described by Pironon et al. (2001), focusing a laser anywhere within a burned phytolith to produce Raman peaks characteristic of graphite carbon, seemed straightforward and the results definitive, replicating most of their results proved at first very difficult and in the end impossible.

The first issue addressed using the UC-Davis Raman instrument was overcoming powerful background fluorescence inherent in individual phytoliths to obtain the much weaker Raman signal. Because of the possibility that the bleach extraction treatment was causing the high background, several additional phytolith extraction procedures as well as simple surface enhanced Raman techniques (aluminum foil treated with iodine) were tested, but almost all heated phytoliths examined still did not produce a Raman signal for carbon, and only a handful produced the Raman silica peaks expected due to the fact that phytoliths are largely composed of amorphous silica (Watling et al. 2011; Pironon et al. 2001). Pironon (personal communication) suggested that he had used a relatively obscure sulfuric acid-based extraction procedure (Kelly 1990) to reduce background; this procedure did a good job of reducing background but the Raman signal of all except obviously darkened burned phytoliths was still too weak to be detected on the UC-Davis instrument.

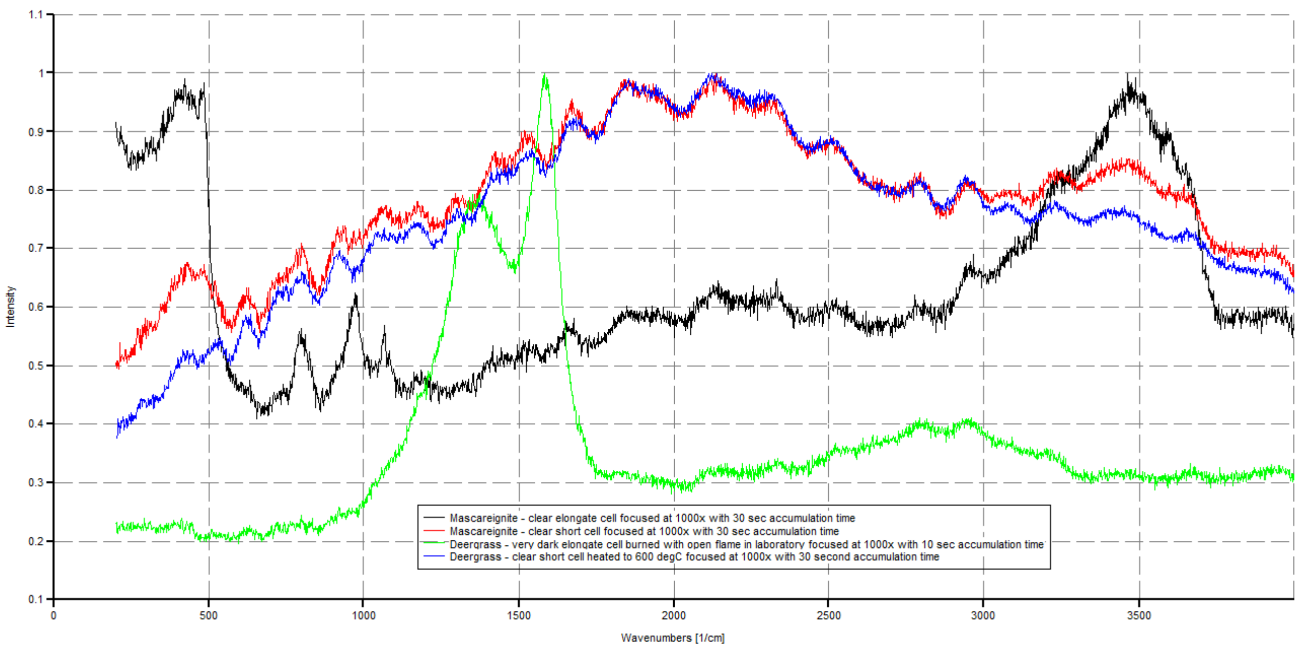
Using the more sensitive confocal Raman instrument located at UC-Riverside, background influorescence of most samples was overcome by adjusting the size of the confocal hole. However, problems persisted with obtaining a strong enough Raman signal to produce silica peaks in the spectra. Personal communications with Jeff Parr and Rosa Albert (phytolith researchers in Australia and Spain, respectively, who have studied Raman spectra of phytoliths) confirmed that they had similar problems.

The Raman spectra of most of the phytoliths examined had rounded peaks that appeared to be artefacts of the instrument (Figure 6, red and blue lines). These peaks were present in all samples (but not aluminum blanks or air) and do not appear to be correlated with phytolith silica or carbon. Phytoliths that were heated in a muffle furnace to create a temperature gradient from 20 to 600°C with the expectation that heat would alter their silica or graphite peaks showed no reliable changes to their Raman spectra. Heating did not generally darken the phytoliths and rarely caused any change to the phytolith spectra. Focusing on a visible piece of occluded carbon inside a heated short cell produced the expected graphite peaks in only 2% of the heated phytoliths examined. Also, finding and focusing on small blobs of occluded carbon in short cells was very difficult.

Visually dark phytoliths of plant leaves that were burned in the laboratory as well as those collected in the field after the control burn had the distinctive graphite peaks at wavenumbers 1372 and 1583 (Figure 6, green line). 96% of the phytoliths that appeared visually dark showed the expected graphite peaks. However, tests of adjacent phytoliths, one darkened and the other clear, still in place on a burned leaf fragment and likely heated to similar temperatures, revealed that the dark phytolith had the graphite peaks, but the clear phytolith did not. This suggests the graphite peaks are not due to heating of occluded carbon inside the phytolith but rather to carbon adsorbed to the phytolith surface during combustion.

A sample of mascareignite, a mineral assumed to have been formed by burning bamboo and found to have graphite peaks by Pironon et al. (2001), was obtained from Pironon. Despite repeated attempts, only the spectra of the small percentage of darkened phytoliths showed the expected graphite peaks; non-darkened phytoliths had no peaks. Only one clear mascareignite phytolith (Figure 6, black line) showed the pattern of peaks expected for silica that was described by Pironon et al. (2001). It is unclear why this particular phytolith showed such a strong signal while others did not. Because heated but not darkened phytoliths did not have graphite peaks, we also looked for subtle shifts in wavenumber that might occur due to heating of the silica in phytoliths. There were no consistent changes in silica peaks at any of the heating temperatures examined.

Figure 6. Raman spectra of individual phytoliths obtained with the confocal Raman spectrometer at UC-Riverside using a 523 nm laser. Red and blue lines are typical spectra of almost all phytoliths examined that were not visually dark. The green line with the distinctive graphite peaks at wavenumbers 1372 and 1583 is typical of spectra of visually darkened phytoliths. The black line is the spectrum of the only phytolith examined that closely matches the silica pattern described by Pironon et al. (2001).



Raman spectra of phytoliths that were not clearly darkened (classified as “possibly burned” and “ambiguous” in the phytolith coloration study) rarely showed any graphite peaks, suggesting that their altered appearance was not due to burning but rather weathering in the soil. It is possible that some of these phytoliths were exposed to fire and darkened with carbon adsorbed to the surface immediately after the fire but biological and chemical weathering of the phytolith surface eventually removed this carbon, leading to an altered phytolith that does not have a graphite Raman signal. Considerably more research is required to understand the effects of weathering on burned phytoliths.

In conclusion, the Raman approach to identify burned phytoliths performed no better than the phytolith coloration approach. Only obviously darkened phytoliths consistently showed the graphite peaks expected from burning. Raman spectra of phytoliths that were heated up to 600°C but with no obvious dark color showed no effects. These results suggest it is not the occluded carbon in phytoliths that undergoes changes with heating but rather the carbon adsorbed by phytoliths exposed to open flames. The Raman approach provides essentially the same results as the phytolith coloration approach, which is much easier to perform and cheaper than Raman spectroscopy.

Pinnacles NP Long-Term Grassland Survey

Soil samples collected from areas in Pinnacles NP that are currently dominated by grasses showed a wide range of soil phytolith percentages (Table 2). Based on previous research in California, sites with soil phytolith percentage >0.30% are considered long-term grass dominated grasslands (Evett and Bartolome 2013). Percentages consistently above this threshold in samples from the large grassland area near the eastern entrance to the park suggest this area was likely dominated by grasses prior to European settlement, not surprising because the relatively high water table there likely contributed to elevated soil moisture that probably favors grasses over forbs in California (Evett and Bartolome 2013). However, samples taken at depth in sediment from cut banks were not as rich in phytoliths, indicating grasses were not as extensive further in the past, although numerous grass phytolith morphotypes were seen. Reduced phytolith percentages at these sedimentary sites may be due to the fact that phytolith input from grasses is diluted by pulses of sediment occurring along the stream channels where the cut banks were found compared to geomorphically stable sites subject to soil formation processes. Phytolith content dilution found in sedimentary environments may also be the case at the extensive, lush meadows at the Northwest Trail picnic ground, where most samples had relatively low phytolith percentages (Table 2), both on the surface and at depth, despite being in an area that likely had a high water table that could have supported the high density of grasses seen today. A 1.2 m cut bank was found just outside the picnic ground along the Cave Trail with substantial phytolith content throughout the upper meter; if radiocarbon dated, this may be a good site for documenting vegetation change.

Surprisingly, many upland sites that are currently CAG patches in a matrix of other vegetation along the High Peaks Trail and the Bushwack Saddle grass patch showed elevated phytolith percentages, suggesting they have been stable and grass-dominated for hundreds to thousands of years. These sites appear quite xeric, which should favor forbs or shrubs, although there may be additional rainfall from orographic effects at higher elevations or edaphic effects that are not readily visible.

Table 2. Soil phytolith content of selected sites currently dominated by grassland at Pinnacle NP. Sites with soil phytolith content >0.30% are considered long-term grass dominated grassland. Samples were also collected from depth at sedimentary sites to possibly provide insight into vegetation change through time. CAG is California Annual Grassland. Precise site location coordinates were not recorded.

Site Location	Surface Vegetation	Sample Depth	Soil Phytolith Content (%)
Deergrass site in McCabe Canyon (mean)	Deergrass	Surface	0.42
Channel below deergrass site in McCabe	Scattered deergrass, CAG	Surface	0.31
Channel below deergrass site in McCabe	Scattered deergrass, CAG	50 cm in cut bank	0.20
Grassland near eastern entrance to NP	CAG	Surface	0.28
Grassland near eastern entrance to NP	CAG	50 cm in cut bank	0.11
Grassland near eastern entrance to NP	Leymus patch	Surface	0.52
Grassland near eastern entrance to NP	Leymus patch burned 2009	Surface	0.73
Grassland near eastern entrance to NP	CAG, very few natives	Surface	0.78
Grassland near eastern entrance to NP	CAG, very few natives	25 cm	0.18
Grassland near eastern entrance to NP	CAG, very few natives	200 cm	0.04
Near eastern entrance gatehouse	Rushes, burned 2009	Surface	0.51
Near eastern entrance gatehouse	CAG	Surface	1.04
High Peaks Trail - ~0.50 miles from W end	chamise, grass	30 cm in colluvium	0.02
High Peaks Trail - ~0.75 miles from W end	Mixed oak, pine, grass	30 cm in colluvium	0.93
High Peaks Trail - ~1.0 miles from W end	Blue oak, pine, grass	30 cm in colluvium	0.16
High Peaks Trail - near top	CAG, chaparral, blue oak	Surface	0.65
High Peaks Trail - grass patch near top	edaphic CAG, some Stipa	Surface	0.82
High Peaks Trail - grass patch east of trail	CAG	Surface	0.60
High Peaks Trail - near top, east side	CAG, but in Stipa patch	Surface	1.05
Northwest Trail picnic site - lower meadow	10 m E at edge of grassland	Surface	0.14
Northwest Trail picnic site - lower meadow	E bank in channel	30 cm in cut bank	0.10

Northwest Trail picnic site - lower meadow	W bank of channel, rushes	Surface	0.71
Northwest Trail picnic site - lower meadow	W bank in channel	30 cm in cut bank	0.10
Northwest Trail picnic site - lower meadow	W bank in channel, CAG	50 cm in cut bank	0.08
Northwest Trail picnic site - lower meadow	5 m W of channel	Surface	0.58
Northwest Trail picnic site - lower meadow	15 m W of channel	Surface	0.17
Northwest Trail picnic site - lower meadow	35 m W, edge of CAG area	Surface	0.11
Northwest Trail picnic site - upper meadow	East bank in channel, rushes	Surface	0.18
Northwest Trail picnic site - upper meadow	East bank in channel, rushes	50 cm in cut bank	0.08
Northwest Trail picnic site - upper meadow	0.5 m W of channel	Surface	0.09
Northwest Trail picnic site - upper meadow	5 m W of channel	Surface	0.21
Northwest Trail picnic site - upper meadow	15 m W of channel	Surface	0.11
Northwest Trail picnic site - upper meadow	25 m W of channel	Surface	0.12
Northwest Trail picnic site - upper meadow	45 m W, edge of CAG area	Surface	0.48
Northwest Trail picnic site - upper meadow	100 m W on hillside, CAG	Surface	0.15
Cave Trail near picnic site	CAG, pine	Surface	0.17
Cave Trail near picnic site	CAG, pine	40 cm in cut bank	0.31
Cave Trail near picnic site	CAG, pine	80 cm in cut bank	0.32
Cave Trail near picnic site	CAG, pine	120 cm in cut bank	0.08
Campground - east side near toeslope	Leymus patch	Surface	1.03
Lowland terrace between 2 channels	Valley oak	Surface	0.11
Bushwack saddle grass patch	CAG, some Stipa, blue oak	Surface	0.47
Bushwack grassland patch	blue oak, coast live oak, CAG	Surface	0.20

Finally, the mean phytolith content at the deergrass site in McCabe Canyon (mean of 12 plots sampled) was 0.42%, including the presence of many saddle short cells diagnostic of deergrass, indicating this site has been stable and dominated by deergrass for hundreds to thousands of years.

IV. Key Findings

1) None of the approaches tested in this study and purported in the literature to distinguish individual burned phytoliths from unburned phytoliths were reliable enough to be useful to accurately estimate past grassland fire regimes in typical soil conditions
   a. The Raman spectroscopic approach, distinguishing individual burned phytoliths through heat-induced changes to occluded carbon, was only able to identify phytoliths that were visually darkened from burning in an open flame, suggesting the carbon measured by the Raman instrument is externally adsorbed during a fire rather than internally altered by heating
   b. The refractive index approach, distinguishing heated or burned phytoliths through changes in the RI due largely to decreased water content, is dependent on fire intensity and not accurate at the relatively low temperatures typical of grassland fires, as well as sensitive to rehydration and phytolith aging effects
   c. The phytolith coloration approach, distinguishing burned phytoliths through their altered color, successfully identified as burned the vast majority of phytoliths from plant leaf material burned with an open flame, both in the lab and from a recent prescribed burn in the field. Phytoliths that were heated in a muffle furnace to grassland fire temperatures but not exposed to direct flame could not be distinguished. However, there were few obviously darkened soil phytoliths found at the deergrass site in McCabe Canyon or at other grassland sites sampled in Pinnacles NP, or indeed at any of the hundreds of grassland sites examined for phytoliths anywhere in California, even at sites expected to have had a long history of frequent native burning. Instead, many soil phytoliths have altered, rather than darkened, color and/or texture. This suggests that darkened, burned phytoliths may be visually altered, either becoming slightly yellowed, light brown, more opaque or with granular texture as they weather after they are deposited in the soil. This approach holds some promise for reconstructing grassland fire regimes if weathered burned phytoliths can be reliably distinguished from weathered unburned phytoliths; considerable additional research is required to determine whether this is possible.

2) Most areas in Pinnacles NP that are currently covered with California Annual Grassland were likely grass-dominated grassland prior to European settlement.
3) Temperatures achieved during a prescribed burn of a grassland plot dominated by deergrass were highly variable and patchy, ranging from unburned to ~700°C.

V. Management Implications

1) Because it is not yet possible to use phytoliths or any other paleoecological approach as a proxy to determine grassland fire regimes in Pinnacles NP prior to European settlement, ethnographic information and the accounts of early explorers to the region, indicating that grassland fires were frequently intentionally burned, remain the most accurate sources of prehistoric fire regimes and should remain the guide for current grassland management.
2) High phytolith content in soils currently covered by grasslands in Pinnacles NP suggests these areas supported grass-dominated grasslands prior to European settlement. Since native grasses were dominant, it is highly appropriate to attempt to restore native grasses and forbs to areas in the park currently dominated by CAG vegetation.

VI. Relationship to Recent Findings and Ongoing Work on this Topic

Since the technique was first suggested almost 30 years ago (Piperno 2006), visual identification of burned phytoliths haas been increasingly used in the literature to document fire in a variety of environments (Kealhofer 1996; Boyd 2002; Gu et al. 2008; Morris et al. 2010). However, no researchers to date have adequately addressed the issue of distinguishing burned from unburned phytoliths in a typical soil phytolith assemblage that includes phytoliths with a wide range of ages and degree of weathering. Publications associated with this research usually has a photograph of a typical darkened phytolith that appears exactly like phytoliths extracted in our study from ash resulting from laboratory burning and prescribed burning of leaf material. Unfortunately, very few phytoliths found in California soils fit this idealized image. It is not clear whether current researchers are counting only those phytoliths that are clearly darkened or whether they are also counting those phytoliths that have some alteration of color or texture. The inability to confidently and reliably identify a burned phytolith in a soil assemblage was a big stumbling block for this study; it is difficult to believe previous researchers did not face similar challenges. There needs to be an explicit examination of this issue, including more research to clarify which weathered phytoliths should be counted as well as a survey of previous authors to determine which altered phytolith types were included in their counts, with publication of results.

Recent research with sponge spicules (Wyche 2012) has highlighted problems with using the refractive index to reliably identify heating of amorphous silica at temperatures below 700°C. At the same time, this research suggests there may be predictable changes, not related to water content, occurring to the fluorescence spectra of biogenic silica when exposed to lower temperatures.

VII. Future Work Needed

1) To make the phytolith coloration approach viable for reconstructing past fire regimes by using fire-altered soil phytoliths, considerable research must be done to document changes that occur in burned phytoliths compared to unburned phytoliths due to weathering and aging in the soil environment. In every soil phytolith assemblage examined, many phytoliths show substantial changes in color, opacity, and texture but very few appear darkened in the same manner as those observed in recently burned leaf material. It is suspected that some of these phytoliths are burned and weathered, but there is currently no method to reliably distinguish them from unburned, weathered phytoliths. If this method is developed, the approach outlined in this study to estimate past fire regimes based on the proportion of burned phytoliths in the soil phytolith assemblage should be viable.
2) The results of the Raman portion of this study should be reconciled with the results of the Pironon et al. (2001) study. This will require a trip to France to observe Pironon's procedures. Phytoliths extracted from samples collected for this study can be brought to Pironon (who is still involved with Raman research but has not worked with phytoliths in more than 12 years) in France to run on his Raman instrument to see if it is possible to replicate his published results using our samples. If the results can be replicated and it is determined how to achieve positive results on our instruments, the Raman approach, which has a sound theoretical basis if there is enough occluded carbon in phytoliths to be detected, could be resurrected and applied as envisioned to reconstruct past fire regimes.
3) Other spectroscopic approaches that could distinguish a heated and/or burned phytolith from an unheated phytolith should be tested. While FTIR spectroscopy is probably not useful for this purpose because the signal is dependent on phytolith water content, approaches such as fluorescence spectroscopy should be investigated further (Wyche 2012)
4) Quantitative morphotype analysis of soil phytoliths can potentially provide species specific information, particularly for grasses, that can be used to determine which species to target when attempting to restore pre-European settlement grassland plant communities in Pinnacles NP and elsewhere.

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