APPENDIX D:
PHYTOLITH AND BIOGENIC SILICA ASSESSMENT OF SELECT SEDIMENT SAMPLES FROM 41BL278

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APPENDIX D: PHYTOLITH AND BIOGENIC SILICA ASSESSMENT OF SELECT SEDIMENT SAMPLES FROM 41BL278

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D.1 SUMMARY

Four sediment samples were processed for phytolith recovery and analysis. Overall phytolith preservation was very poor due to the basic pH environment of the Venus clay loam soil matrix which resulted in extensive phytolith chemical weathering with apparent preferential dissolution of the smaller but very important Poaceae short cell phytoliths. Bulliform cell phytoliths did survive, but generally were very weathered. No diatoms or statospores were observed, although sponge spicules were recovered from all four samples. Carbonate deposits containing root impressions were present in the sand fraction, emphasizing the calcic nature of the soil. The calcic soil preserved snail specimens which were recovered.

D.2 INTRODUCTION TO PHYTOLITHS AND BIOGENIC SILICA

Phytoliths form as a byproduct of plant water transport. When soil pore water is absorbed by plant roots, silicon along with other dissolved ions also enter and are distributed throughout the plant. As water is lost via evapotranspiration from the leaves, silicon becomes trapped in the plant cells. As silicon accumulates in the cells, it gradually solidifies and in many cases is preserved in the shape of the plant cell in which it formed. When the plant dies and decays, the inorganic silicon--in the form of $SiO_2 \bullet nH_2O$--becomes a mineral component of the soil's silt fraction. Thus, the assemblage of siliceous plant cell casts, or phytoliths, become a microfossil record of the plant community living in that location at that time. (Pearsall 2000; Piperno 1988, 2006).

The same precipitated silicon species also occurs in several other organism types which--when recovered in soil are generally referred in aggregate as biogenic silica. Besides phytoliths, of the other forms (i.e., siliceous sponge spicules, statospores, diatoms, and radiolarians)--only sponge spicules were recovered from 41BL278.

Although the various forms of biogenic silica are chemically the same, they may vary in their water content (which is denoted by the "*n*" in the above formula). This matrix water variation in turn leads to variation in particle density, with a density range from about 1.50 to .30 g/cm^3 (Piperno 2006:15). This particle density difference does not affect biogenic particulate recovery as the heavy liquid solvent used to recover the phytoliths and other biogenic silicas is 2.35 g/cm^3, so the entire range of lighter particle densities are recovered together in a single fraction. However, variation in phytolith density is extremely important in soil particle stability as different density particles dissolve at different rates when incorporated in a caustic pH environment (Iler 1979:46-47; Sudbury 2014a). It turns out this pH sensitivity is very important in the sediment samples examined from 41BL278.

D.3 SAMPLE PREPARATION

The samples (Table D - 1) were transferred to 250 milliliter glass jars (Figure D - 1), oven dried, and dry soil weights obtained. The samples were then shaken vigorously in a sodium hexametaphosphate solution [a discontinued Calgon® formulation] for 24 hours. The samples were allowed to settle and come to room temperature, and then the initial mixture of suspended silt and clay was removed by decanting into a settling columns. Then, using settling times calculated via Stoke's Law, each sand-containing

Table D-1. Sediment Samples from 41BL278.

JSE Lab Sample Number	MQ's Sample No.	Block	Unit	Level	Depth (cmbs)	Feat. No.	PNUM	Cat. No.	Ext. No.	Comment
MQ14-6	1	BT 8	2	16	152-155		0008	004	1b	control
MQ14-7	2	BT 9	5	14	143	3A	0503	004	1a	S4; N13 E89; from under BR4
MQ14-8	3	BT 9	7	18	173	7	0507	004	2a	S2, N30 E51; from under BR2
MQ14-9	4	BT 9	6	17	165	8	0508	004	1a	S1; N35, E95; from under BR1

sample fraction was repeatedly remixed, allowed to settle, and decanted until all of the silt and clay components were decanted and pooled in settling columns. The clean sand fractions were then oven dried, weighed, and photographed (Figure D-2) and transferred to glass Petri dishes for later examination (Figures D-5).

After three days initial settling, ~80% of each suspended clay fraction in the silt/clay mixture was decanted into another settling vessel. The residual clay/silt from the bottom 20% of each mixture was then quantitatively transferred to a 250 glass jar for the remaining particle separation steps. Again using particle settling times based on Stoke's Law, regular remixing and decants of the suspended clay (~80% of the mixture volume) were removed and pooled for later retrieval and examination. After the liquid phase over the settled silt fraction was clear, the final liquid removal was performed by aspiration in order to remove as much water as possible without disturbing the sediment bed (Figure D-6). A white upper layer of variable thickness-- presumed to contain carbonate-- was visible in all settled sample silt fractions. Rather than dissolving the carbonate at this stage of preparation, the samples were oven dried, weighed, transferred to crucibles for organic constituent removal, and treated in a muffle furnace at 535ºC overnight to remove organic materials.

Next, the ashed silt fractions were transferred to 50 ml plastic centrifuge tubes. The lower density phytolith fractions were separated from the quartz-based silt matrix using a 2.35 g/cm^3 zinc bromide solution. The recovered lower density particulate fraction was pooled for each sample, and once sequential flotations of the parent silt sample did not produce any more particulate material, water was added to the isolates to dilute the liquid density to below 1.50 g/cm^3, and the particulate was recovered via centrifugation. The centrifuge pellets were rinsed multiple times to remove residual zinc bromide, and then oven dried and weighed. Hydrochloric acid (10%) was used to test for carbonate carryover into the isolated phytolith fraction (Figure D-7) Carbonate was present, so all sample phytolith fractions were acid treated, remixed, centrifuged, and the clear acid phase removed via Pasteur pipet until no more microscope slides of the phytolith isolates were prepared mounting 1-2 mg of sample phytolith/biogenic silica isolate in Canada Balsam as illustrated elsewhere (Sudbury 2011a:50-53). The slides were cured in a 35ºC incubator for several weeks, at which time the balsam at the cover slip edges had set sealing the slides. The slides were then scanned at 500x while counting particles and rolling particles as needed for clearer discernment. Due to low phytolith counts and obvious poor particle preservation (Table D-2), the slides were also rescanned entirely at 100x to search for significant specimens that may have been overlooked if they had been between 500x scan transects. A number of sponge spicules and spicule sections were noted during the 100x scans.

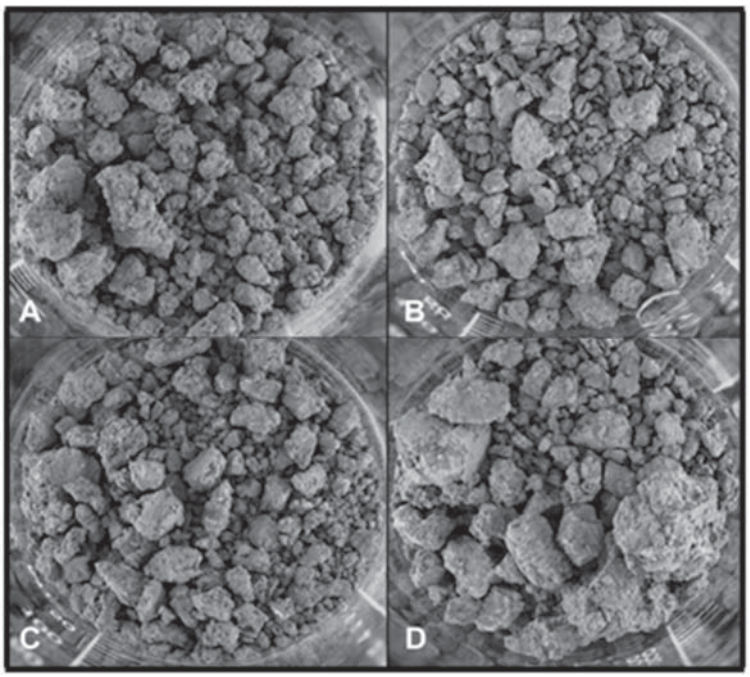

Figure D-1. Oven dried soil samples before disaggregation (41BL278). A = Sample 6; B = Sample 7; C = Sample 8; and D = Sample 9 (Sample identities in Table D-1).

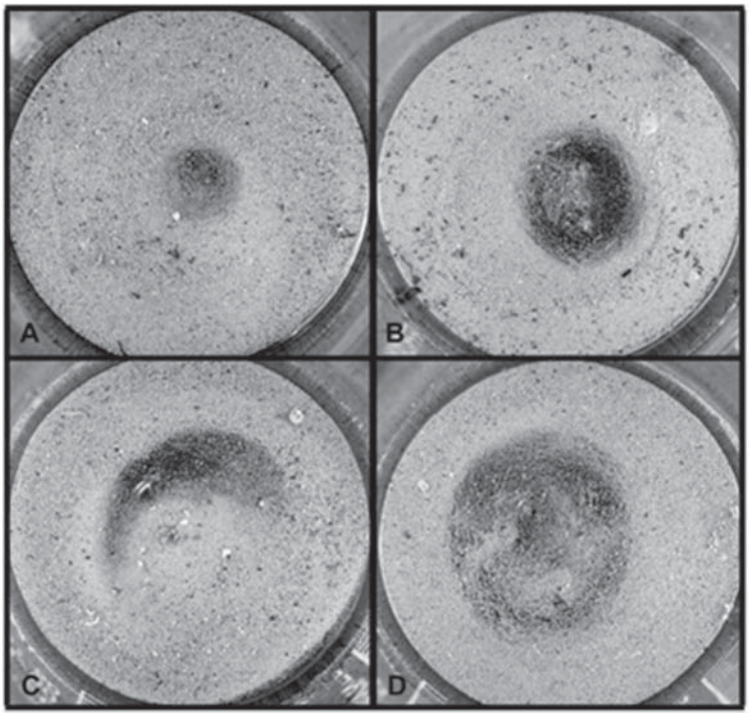

Figure D- 2. Isolated sand fractions (41BL278). A = Sample 6; B = Sample 7; C = Sample 8; and D = Sample 9 (sample identities in Table D-1). [Jar internal diameters are 2.2 inches. The domed glass bottoms show in the center of the jars. The faint yellow marks in C and D are reflection of the camera flash.]

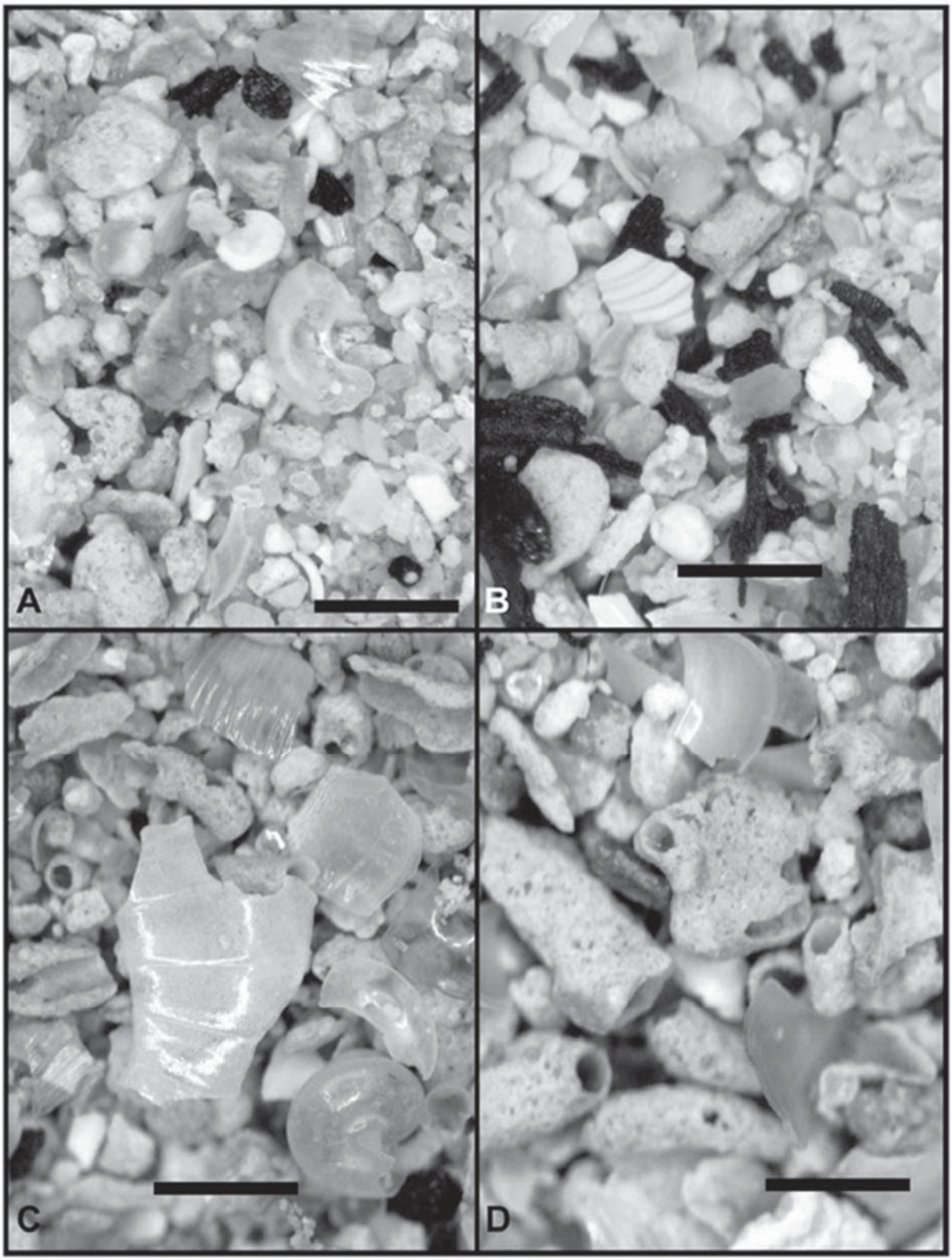

Figure D-3. Carbonates, snails, charcoal, and other particles present in the sand fractions (41BL278). A = Sample 6; B = Sample 7; C = Sample 8; and D = Sample 9 (Sample identities in Table D-1). Bar scales 1 mm.

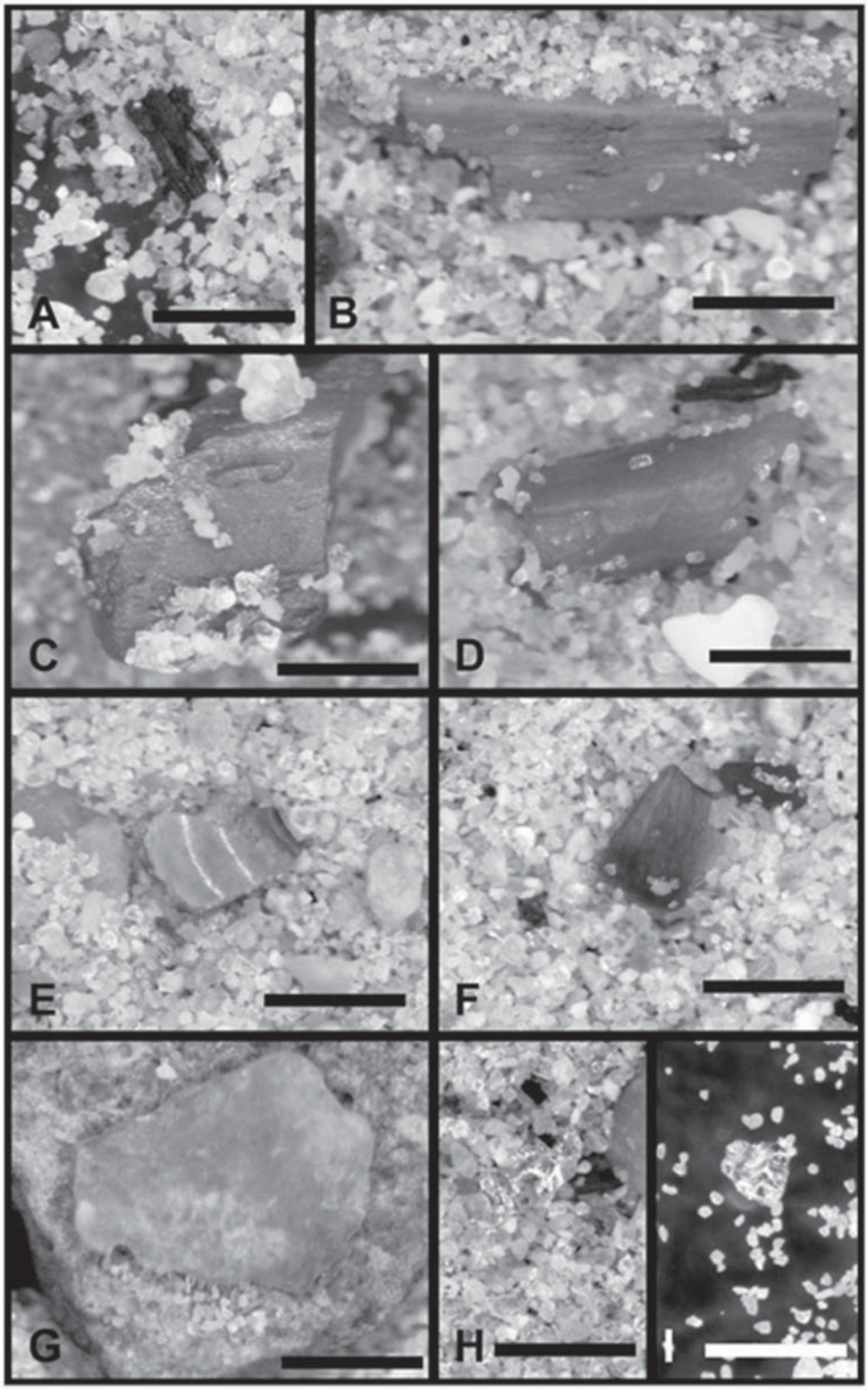

Figure D- 4. Debris observed in the 41BL278 sand fractions (charcoal, bone, burned shell, and lithics). A = charcoal; B-D = bone, E-F = burned shell, and G-I = lithic material. Sample 6 = A-C; Sample 7 = D-H; and Sample 9 = I (Sample identities in Table D-1). Bar scales are 1 mm.

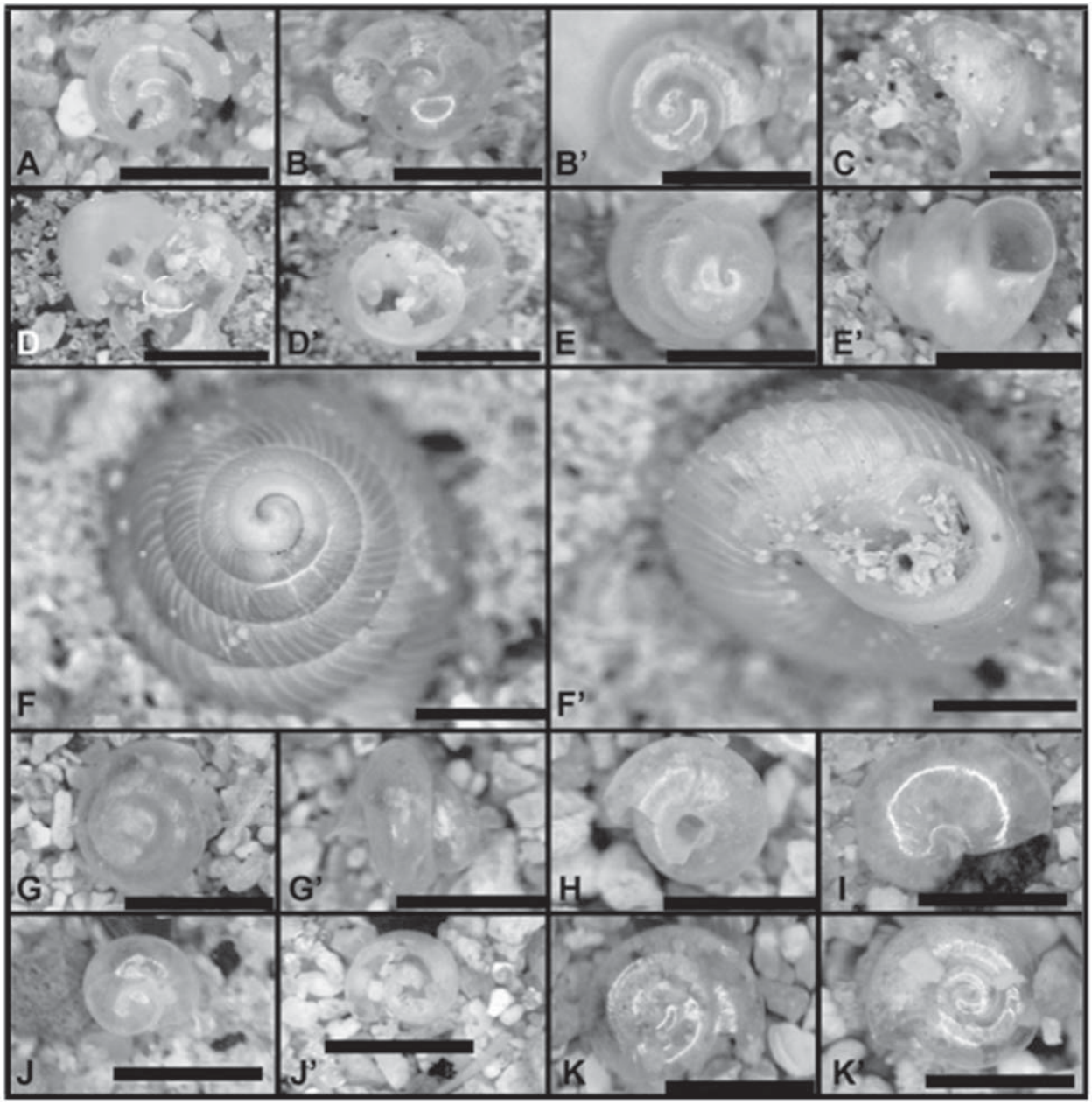

Figure D-5. Snails recovered from the 41BL278 sand fractions shown in Figure D-2. A-D = Sample 6; E = Sample 7; F-H = Sample 8 [specimen F is visible in Figure D-2C at ~1:30 near the jar wall; specimen G is also illustrated in Figure D-3C, bottom right corner], and I-K = Sample 9 (Sample identities in Table D-1). Bar scales are 1 mm.

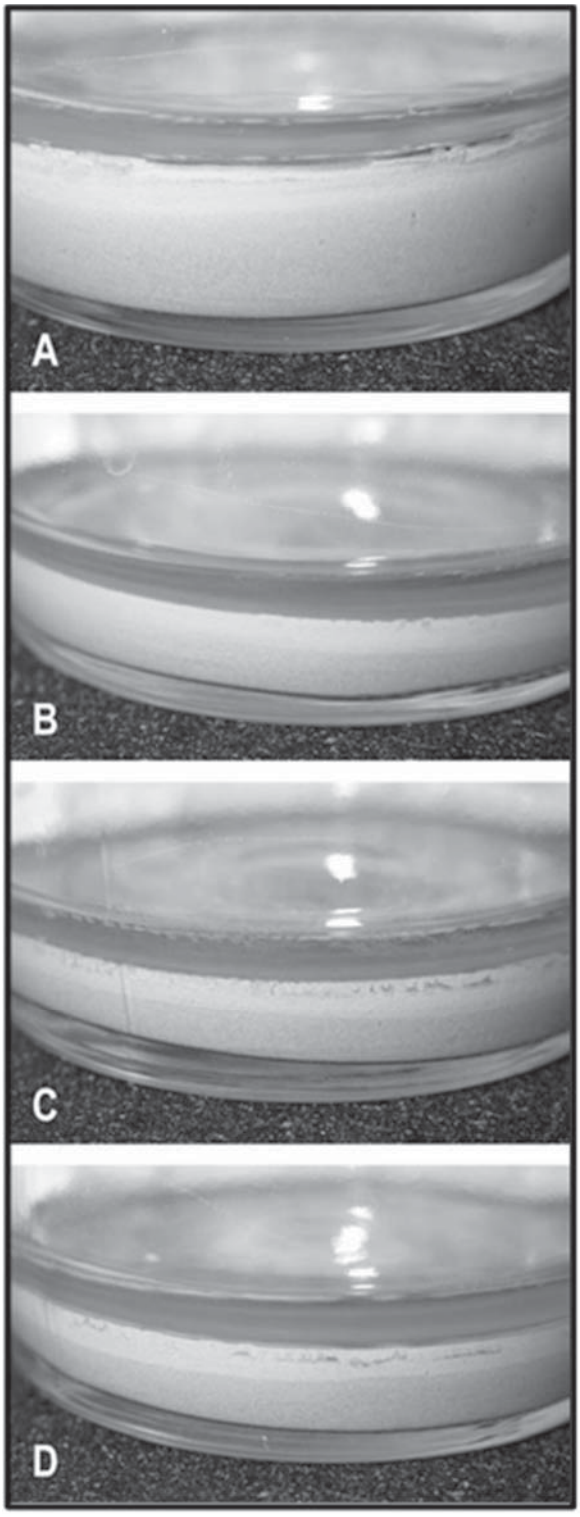

Figure D - 6. Silt sample fractions after clay known to be removal (41BL278). The top gray layer is due to the presence of residual water. The lower gray layer is quartz silt, and intermediate lighter layer is carbonate. A = Sample 6; B = Sample 7; C = Sample 8; and D = Sample 9 (sample identities in Table D-1).

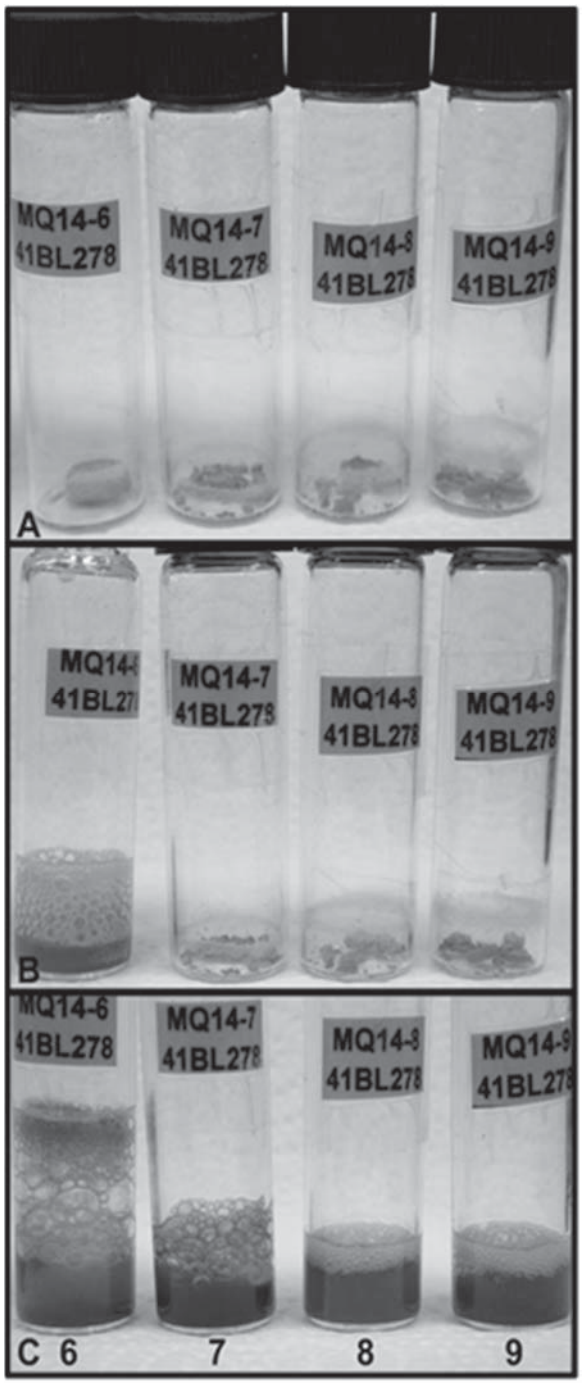

Figure D-7. Carbonate removal from mination performed here, so that phytolith fractions in 8 ml vials (41BL278). Difference may have adversely affected A = dried sample phytolith fractions from the textural determination. Very low flotation. B = hydrochloric acid added to phytolith concentrations was present. Sample 6. C = hydrochloric acid added to all samples showing effervescence (sample identities in Table D-1).

D.4 RESULTS

D.4.1 Sand Fractions

The four sample over-view shots in Figure D-3 show the general range of sand-size particle types observed in these samples. After quartz sand, the most abundant component appeared to carbonate. In all samples, evidence of at least a portion of the carbonate deposits having formed around roots was apparent (most readily seen in Figure D-3D). There were also abundant shell (most likely all from snails) and charcoal, as well as some burned shell and bone fragments, as well as lithic debris (Figure D-4) and whole snail specimens (Figure D-5).

Figure D-4 shows examples of some of the cultural debris observed in the sand fractions: charcoal (Figure D-4A), bone fragments (Figure D-4B-D), burned shell fragments (Figure D-E-F), lithic material and other stone (Figure D-G), and possible quartz microflakes (Figure D-4H-I). Although some of the apparent pieces of quartz present in the samples looked like shatter (angular pieces of variable thickness), these micro-specimens appeared to have bulbs of percussion on them. Due to clarity and small size, the quartz flakes are very difficult to locate, examine, and to photograph.

D.4.2 Silt Fractions

After the clay was removed from the silt by decanting and aspiration, the remaining silt had two strata beneath the residual water (Figure D-6 [color variation may possibly be due to camera settings]). In all cases, there is a lower darker colored layer (grayish), with an upper light colored near-white layer. Although both layers are components of the silt fraction--which is a definition based on particle size--the darker bottom portion is quartz-based silt, and the upper lighter portion [likely finer particles so they settle more slowly in the silt fraction] is predominantly carbonate. This carbonate is the "fine" version of the same abundant material noted in the nodular carbonate fragments observed in the sand fractions (Figure D-2). Although calcium carbonate (2.83 g/cm3) is denser than quartz-based sand (2.65 g/cm3), the carbonate [and possibly other minerals] settled on top of the quartz-based silt as the carbonate is smaller size particles. [Stoke's Law settling calculations are based on the nonrealistic assumptions of sphericity and uniform density--both assumptions which are not 100% correct in this application]. It is probable that there are also larger carbonate particles present in the darker gray zone, but this was not tested.

The most important ramification of these observed layers is that both isolated textural fractions--the sand and silt-- contain a high carbonate content. This --along with the accompanying basic pH-- deleterious to good phyto- survival in a basic pH matrix (Iler 1979:41-47; Piperno 2006:22; Sudbury 2014a).

Carbonate removal was performed on the entire silt fraction in a recent sample suite (Sudbury 2014a), which took a very prolonged time to successfully execute. In processing this current sample batch, the silt fraction--including carbonate-- remained intact following thermal organic removal. The resulting isolates are shown following flotation, phytolith fraction isolation, recovery, and drying (Figure D-7A). Ten percent hydrochloric acid was added to Sample 6 (Figure D-7B) which resulted in effervescence indicative of carbonates having been recovered along with the phytoliths. Thus, the remaining three samples were also acid treated (Figure D-7C). The samples were reacted, centrifuged, the clear spent HCl removed and replaced with fresh HCl, and the sample remixed. These acidification and clean-up steps were repeated until no effervescence resulted. Then the remaining solids were rinsed with distilled water and centrifuged five times to remove any residual acid. Based on weight loss, more than two- thirds of the original isolated "phytolith" fraction weight turned out to be carbonate (Table D-2).

Table D-2. Soil Texture and Phytolith Soil Concentrations (41BL278).

JSE Lab Sample No.	Depth (cmbs)	Feature No.	Sample wt. (g)	Sand wt. %	Silt wt. %	Phytolith Fraction (wt. % of soil)	
						with CO present	with CO removed
MQ14-6	152-155	*control*	47.88	10.2	54.2	0.23	0.07
MQ14-7	143	3A	20.64	11.9	54.2	0.22	0.04
MQ14-8	173	7	28.68	11.6	51.9	0.14	0.03
MQ14-9	165	8	27.18	11.7	52.8	0.08	0.04

Since the carbonate was never removed from the sand fractions, the intact silt and sand fraction weights were recorded as the textural component weights. Gravi-metrically, all four samples are classified as silty clay loams--which is a slightly higher silt content than anticipated based on USDA soil data. However, USDA soil textural analysis is based on hydrometer measurements vs. the gravimetric deter-

All decanted clay fractions were recovered and examined before storage. Three clay fractions were collected based on the settling procedure and container volumes used. The original decant from the two liter bottle containing the original silt/clay mixture decanted from the sand was kept separate from the later decants performed during final removal of the clay from the silt. The material that initially settled from the original decant was also kept separate from the larger volume of the first clay solution. Two of these "settled" clay fractions are illustrated in Figure D-8 due to their strikingly different appearance. The amount of soil sample 6 that was processed was 76% larger soil sample than Sample 9; however, the difference between the Sample 6 clay volume fraction and Sample 9 clay fraction is larger than that difference (Figure D-8). Even more striking are the additional banding of strata present in Sample 6 that are not visible in Sample 9. The cause of this difference has not been determined, but as the total percent clay content of the parent samples is approximately the same, the observed differences are almost certainly at least in part a reflection of differences in clay particle size and/or composition between the two samples. The slight difference in phytolith recovery (preservation status?) between these samples suggests that the samples may also contain different carbonate levels (or at least a different effective soil pH), and/or possible differences in water flow or soil permeability.

D.4.3 Phytoliths

Phytolith recovery was very low (Table D-2), with short cells in particular being in very short supply in all samples [the sample counts were each less than 50 short cells; the bulliform counts were 3-20x higher in each sample]. Not enough

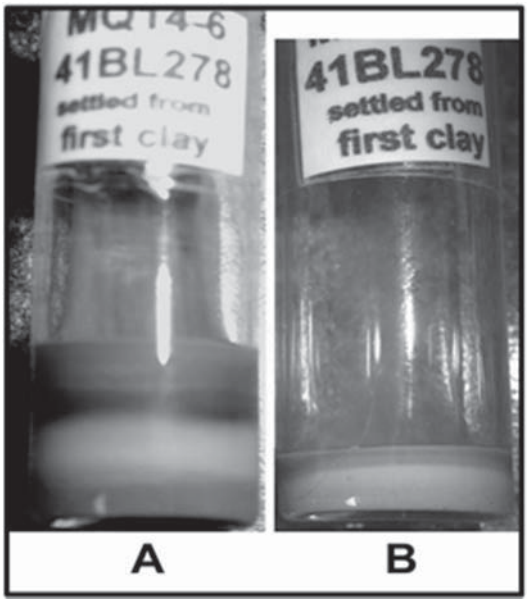

Figure D-8. Sedimentation of decanted clay fractions (41BL278). A = Sample 6; B = Sample 9. (Sample identities in Table D-1).

short cells were observed to reach minimum count required criteria (Strömberg 2009). "Classic" form short cell phytoliths are shown in Figure D-9. The larger crenate forms show some evidence of surface damage that is likely attributable to chemical weathering due to the basic soil environment; this is most noticeable in Figures D-9C, E, and H-J (Sudbury2014a). The smaller panicoid forms with apparent weathering do no show surface pitting nearly as much as what appears to be complete dissolution of parts of the particles (c.f., Figures D-9L-N, P-R, V, and W). Many of the very uncommon Aristida lobate (Figure D-9Z) and saddle phytoliths (Figure D-9BB) also show evidence of what is likely partial particle dissolution.

Although a number of elongate forms were present (Figure D-10D and M), by far the most abundant phytolith form present in the samples were the bulliform cells (Figure D-10). Although some particles were in fairly good state of preservation, most bulliform and elongate cells showed varying degrees of particle damage--again most likely attributable to partial dissolution due to the basic pH environment in the calcic soil. Looking at the specimen in Figure D-10I, it is not hard to envision a root growing adjacent to the particle, and the more basic pH environment immediately surrounding the root contributing to dissolution of the portion of the phytolith that it was in contact with.

Phytoliths generally attributed to trees were in much better condition (Figure D-11). Tracheid elements (Figure D-11A through D), spiny spheroids (Figure D-11E through G), and angular phytoliths (Figure D-11H through R) were all in a good state of preservation. Some of the angular phytoliths show some surface pitting from weathering (Figure D-11I, O, and Q), but overall the tree origin phytoliths are in better shape than the short cell and the bulliform cell phytoliths.

Some of the very large biogenic silica fragments recovered at 41BL278 are more sheet- like and, based on their darkened appearance, show evidence of being burned (Figure D-12); these are likely derived from molten or semi-molten biogenic silica from grass and/or tree origin. Most of these particles (all except Figure D-12G) show more evidence of surface pitting than the specimens in Figure D-11. This enhanced surface pitting is presumably due to chemical weathering in the soil environment; it is more severe in the overheated specimens (Figure D-12) than in any of the tree angular forms (Figure D-11H trough R), but significantly less that observed in the bulliform and elongate cells (Figure D-10A through P). Heating would tend to drive off residual water within the phytolith matrix, increasing its particle density. Thus, this proposed "heating correlates with more chemical weathering" observation based on empirical evidence is in full agreement with the prior observation that higher biogenic particle density is correlated with more rapid particle dissolution in a basic pH solution (Iler 1979:46-47; see also Sudbury 2014a). This effect likely correlates with particle hydration and thus particle density (ibid.). Natural grassland fires do not generally burn hot enough to melt phytoliths, so a hearth or some other cultural burn is more likely to be the cause of this disfigurement.

The specimen in Figure D-13 is even more exciting--although darkly discolored, the outlines of the presumed plant elongate cells are still clearly visible. They are on the lower side of the particle as mounted on the slide (in focus in Figure D-13A). The weathering process appears to have been more severe on the upper side (Figure D-13A') where clear evidence of the cellular structure are no longer visible except as vague hints. If this mass was against the ground when exposed to heat, the ground side would have likely have been cooler than the side exposed to the heat source. The difference in residual visible

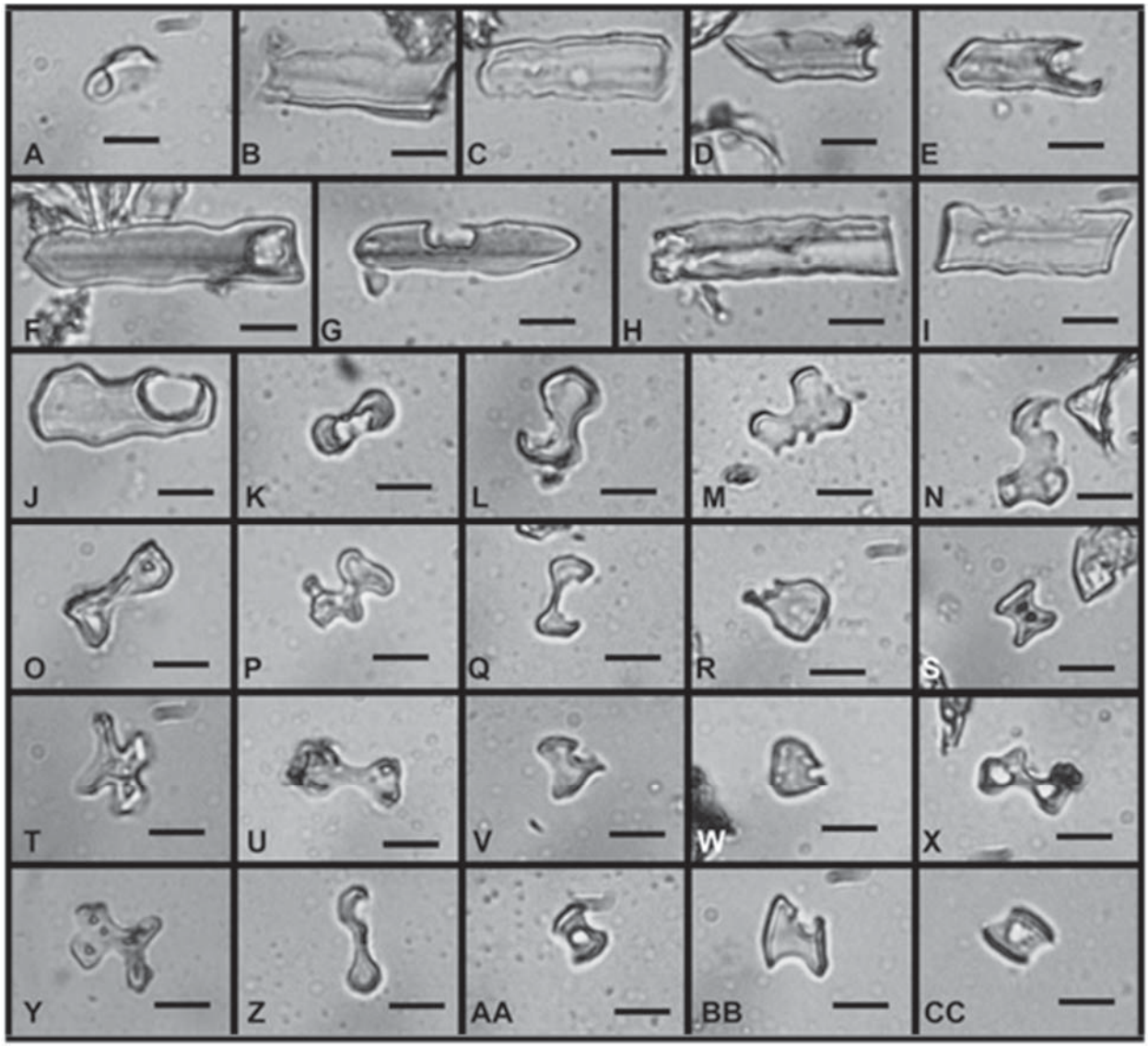

Figure D-9. Short cell Poaceae phytoliths (41BL278). Sample 6 = A-C, F, K-S, and Z-CC; Sample 7 = D-E, G, T; Sample 8 = H, and U-V; and Sample 9 = I-J and W-Y. Pooids = A-J; Panicoids = K-Y; Chloridoids = AA-CC. Image Z is an Aristida lobate specimen. Images E, J, M, N, Q, R, V, W, Z, and BB show damage that appears to be from particle dissolution rather than from mechanical damage/breakage (sample identities in Table D-1). Bar scales are 10 microns.

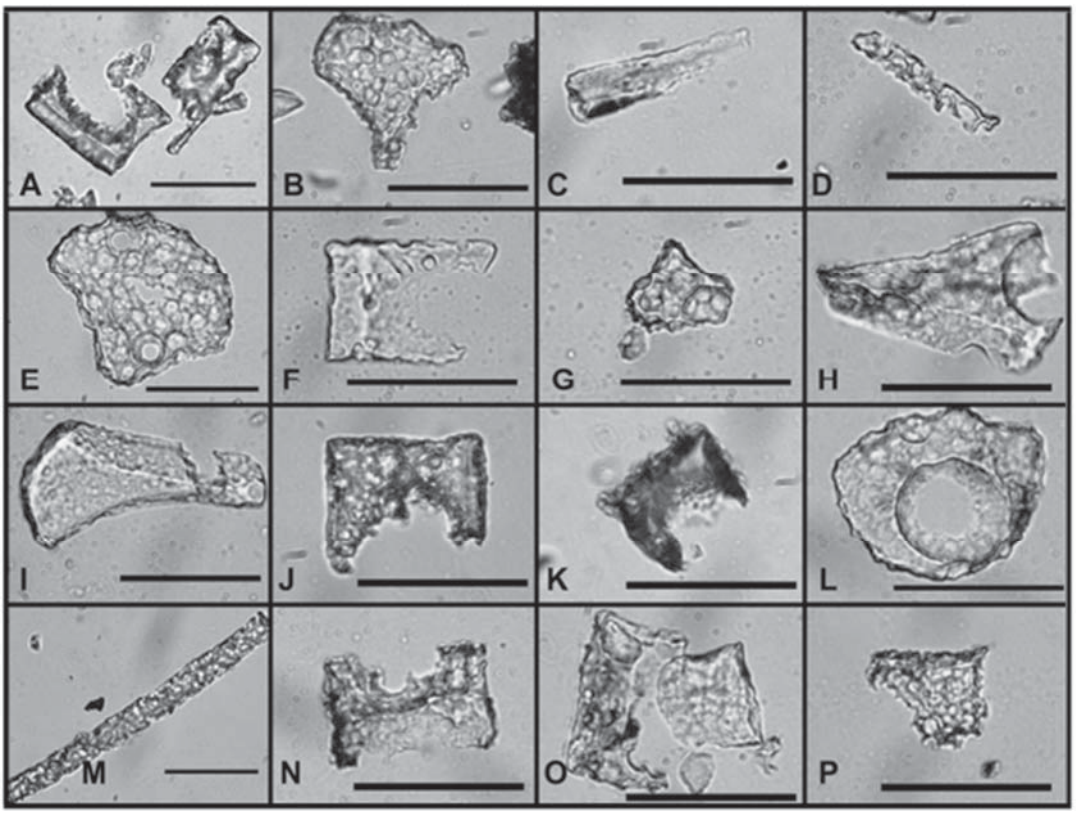

Figure D-10. Chemical weathering of bulliform and elongate phytoliths (41BL278). A = Sample 6; B-D = Sample 7; E-L = Sample 8, and M-P = Sample 9 (Sample identities in Table D-1). Bar scales are 50 microns.

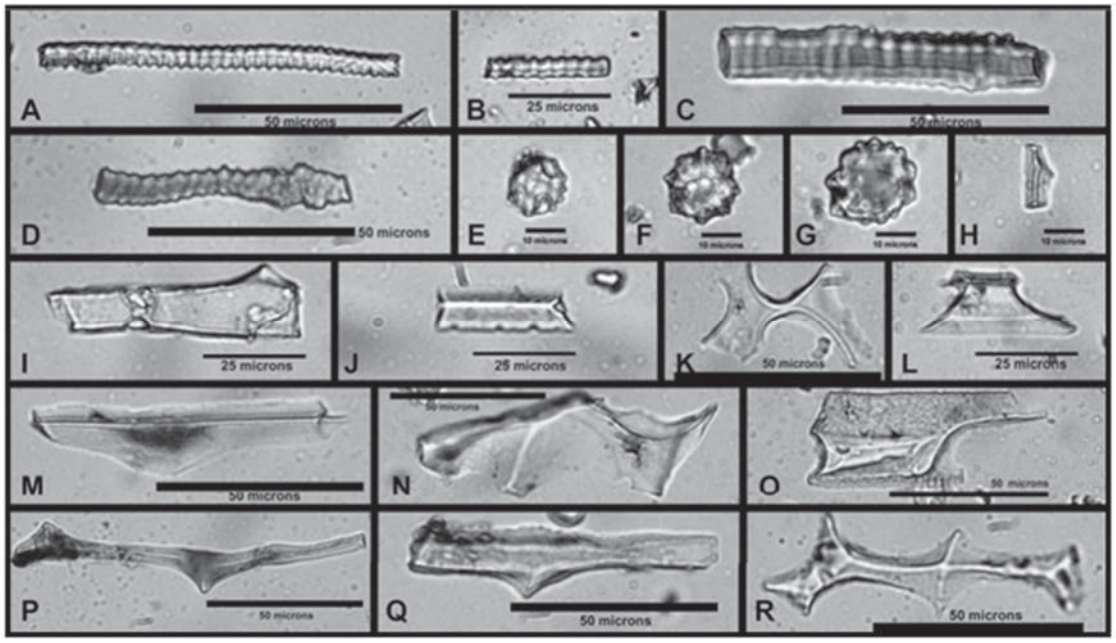

Figure D-11. Tree-related phytoliths (41BL278). Sample 6 = A, B, E-M; Sample 7 = C, N-P; Sample 8 = D, Q; and Sample 9 = R (Sample identities in Table D-1). Bar scales as marked.

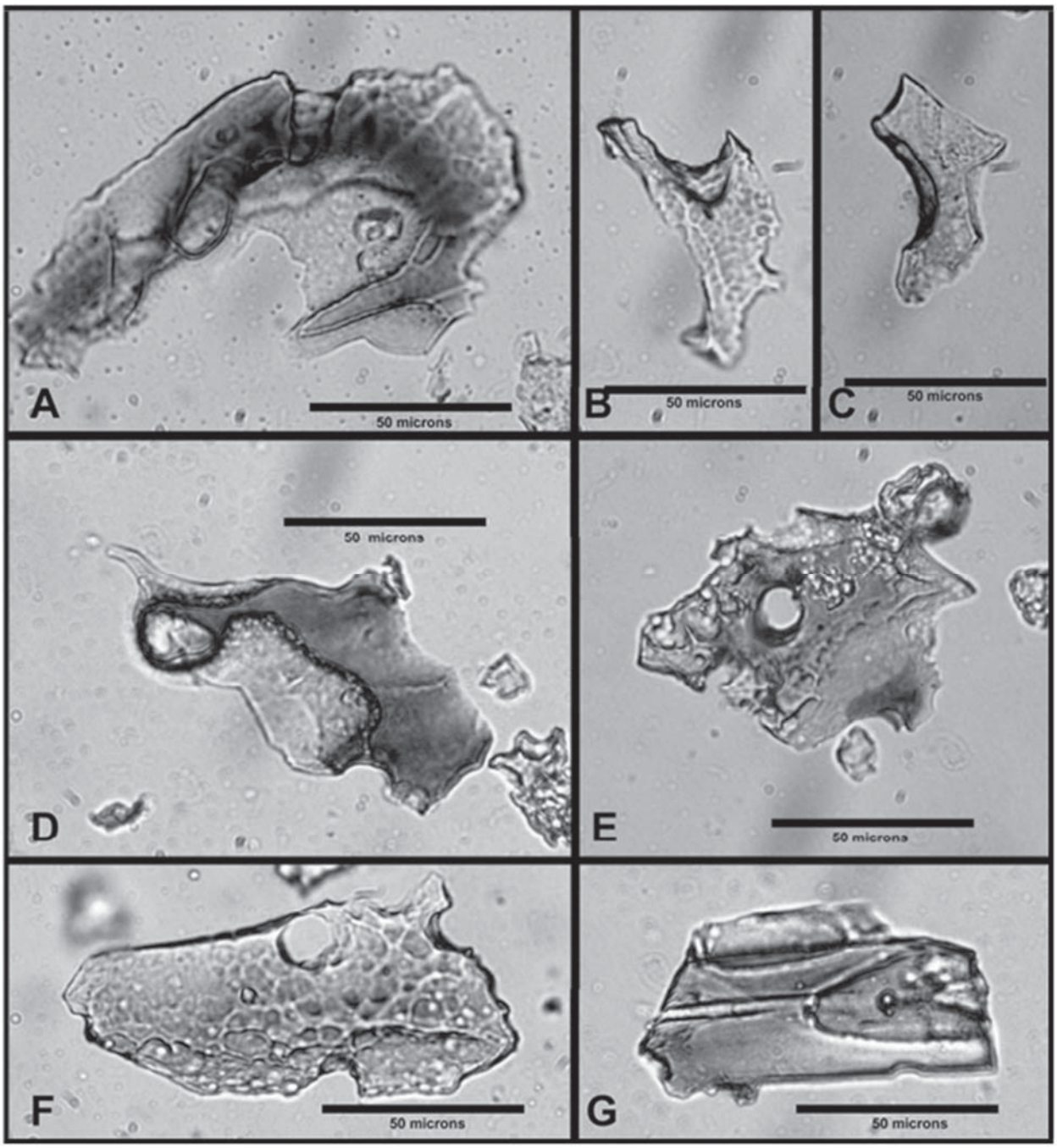

Figure D-12. Burned irregular "sheets" of amorphous silica (41BL278). Sample 6 = A-E; Sample 7 = F; and Sample 8 = G (Sample identities in Table D-1). Bar scales are 50 microns.

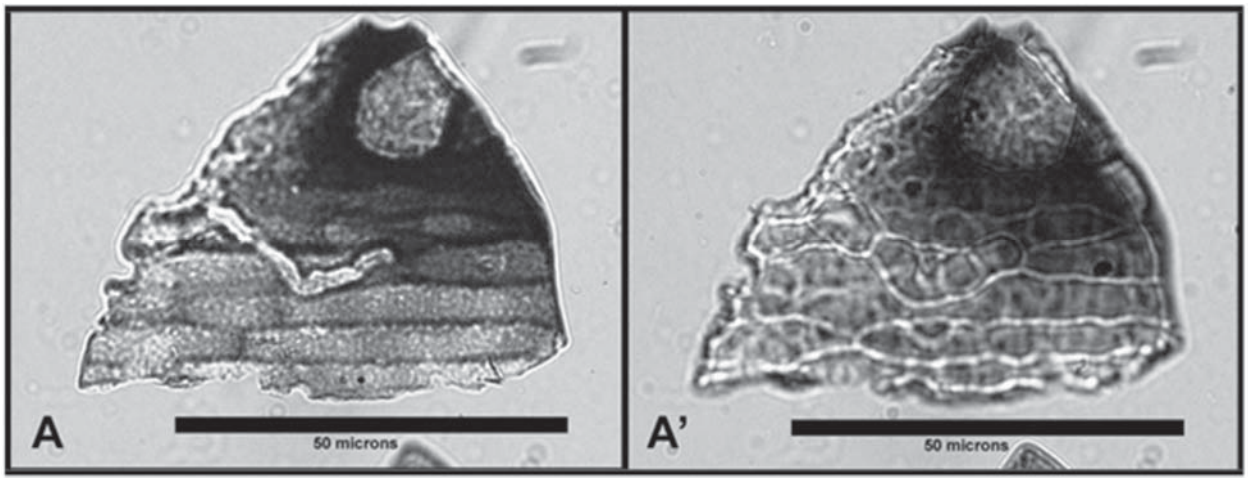

Figure D-13. Burned fused phytoliths (41BL278). The same amorphous silica specimen is illustrated in A and A', and appears likely to be comprised of elongate cell phytoliths. The difference between the two images is the focal plane of the microscope [A is focused on the bottom surface of the tabular particle, and A' is focused on the top surface. Amorphous silica particles are clear--which is why one can view both sides of the particle; however, burning has darkened this particle]. (The specimen in A/A' is from Sample 9; sample identity is in Table D-1).

cellular structure on the two particle faces seems to support this interpretation. A somewhat similar specimen was reported previously--the cells were fused, but their structure was not obscured on either side, the melting was not as extensive, nor was the particle as severely discolored (Sudbury 2014a: Figure 35A and A').

Some of the various unidentified phytolith specimens observed during the course of this study are illustrated in Figure D-14 for future reference.

D.4.4 Other Biogenic Silica Observed

Fresh water siliceous sponge spicules were present in all four samples (Figures D-15 through 17). No gemmoscleres (reproductive spicules) were recovered. Other biogenic silica forms (i.e., diatoms and statospores) were not observed in any of the 41BL278 soil samples.

Complete spicules were recovered from all samples (Figure D-15A, D-16A, B, F, and D-17A and B). None of the complete specimens were weathered suggesting an available local water supply which supported a freshwater sponge population. Weathered specimen sections are present, suggesting movement caused abrasion and/or soil induced chemical weathering (Figure D-15B, G, and H; Figure D-16H; and 17C, D, and E). The other fragmentary specimens not specified do not show evidence of significant surface weathering.

Several of these illustrations are of particles of uncertain identity. The overall appearance of the specimen in Figure D-16D is reminiscent of a spicule, but the internal morphology is somewhat aberrant. More importantly, the rounded closed end does not occur in modern freshwater sponges in North America--all modern specimens are bi-pointed with orifices at each end. Marine glass sponges do occur with a single orifice and a rounded second end--so specimen Figure D-16D may be a fossiliferous spicule. The sharp angular projection on the specimen shown in Figure D-16J suggests it is not a spicule, but may be some other amorphous particle type. Specimen in Figure

D-16J makes one wonder whether or not other large bore thin-walled specimen sections are spicules [Figures D-15E and F; D-16C and I]--either from an aberrant thin-walled version of modern species or an unknown species. The thin-walled specimen reported from 41TV2161 showed the classic enlarged area which housed the sclerocyte--which positively confirmed its identity a spicule (Sudbury 2014a: Figure 28K). This morphologic feature was not present in any of the 41BL278 thin-walled specimens [in the current sample, this feature is most clearly visible in the specimen Figure D-16A; the specimen in Figure D-15A has a thickened outer wall in the center of the spicule, but does not have the enlarged area in the axial canal]. As both the thin-walled and single orifice/round-based specimens are now known from two sites of similar age in the same general geographic area, there is the possibility that they represent new mid-Holocene sponge species.

Another possible explanation for the two aforementioned odd morphologic forms is that they are atypical spicules formed by sponges in response to environmental stresses. The specimen in Figure D-16B almost certainly fits in this category; with its bulbous appearance, it looks more like a serological pipet than a spicule--this is not a typical fresh water spicule morphology. Evaluation of additional sponge spicule samples from this and similar age sites is clearly needed to clarify these issues.

For additional background information about freshwater sponge spicules, consult Reiswig, Frost, Ricciardi (2010), Harrison (1974), or Sudbury (2011c).

D.5 DISCUSSION

Biogenic silica preservation at 41BL278 was poor--no diatoms or statospores were present, short cell phytoliths were observed in very limited numbers, and the more abundant bulliform cells generally showed extensive surface pitting from chemical weathering which is readily attributed to the calcic soil environment. Only the sponge spicule and tree origin biogenic particles showed good preservation.

Phytoliths have been processed at JSE labs from four different Calf Creek affiliated sites in the past four years; a summary of the occupation zone phytolith concentrations is presented in Table D-3. All four sites were located on or very near active water ways when occupied (34WO69 may have been on more of a spring, seep, or marshy area (Barbara Winsborough, personal communication). The two Texas sites are located in central Texas, whereas 34WO69 is near the Oklahoma Panhandle, and 34NW132 is in far northeastern Oklahoma. The Texas sites had calcic or cambic soil soils with high carbonate content, whereas the Oklahoma sites were slightly acidic to near neutral. As can be seen in Table D-3, the total phytolith recovery from the two Texas sites was very low (<0.10 wt. % of soil once the carbonates were removed--and the vast majority of that weight is attributable to the much larger and much more abundant bulliform cells and spicules). In contrast, the soil phytolith concentration was much higher on the Oklahoma sites, both in the cultural zone of interest (Table D-3) and throughout the overall profile. The actual average soil profile phytolith concentration at 34WO69 was 2.58 wt. %, and at 34NW132 was 1.62 wt. % of soil, and no detrimental particle preservation issues encountered at either site. Note that, with a much deeper profile, soil aggradation at 34NW132 in an active flood plain was much more rapid than at 34WO69 which likely accounts for the lower relative phytolith concentration at 34NW132.

The biogenic assemblage (phytoliths, diatoms, sponge spicules, and statospores) from the Opossum Creek Site (34NW132) was reported in detail (Sudbury 2011b). Below the surface control/plow zone interval at Opossum Creek, the profile samples were collected based on visible

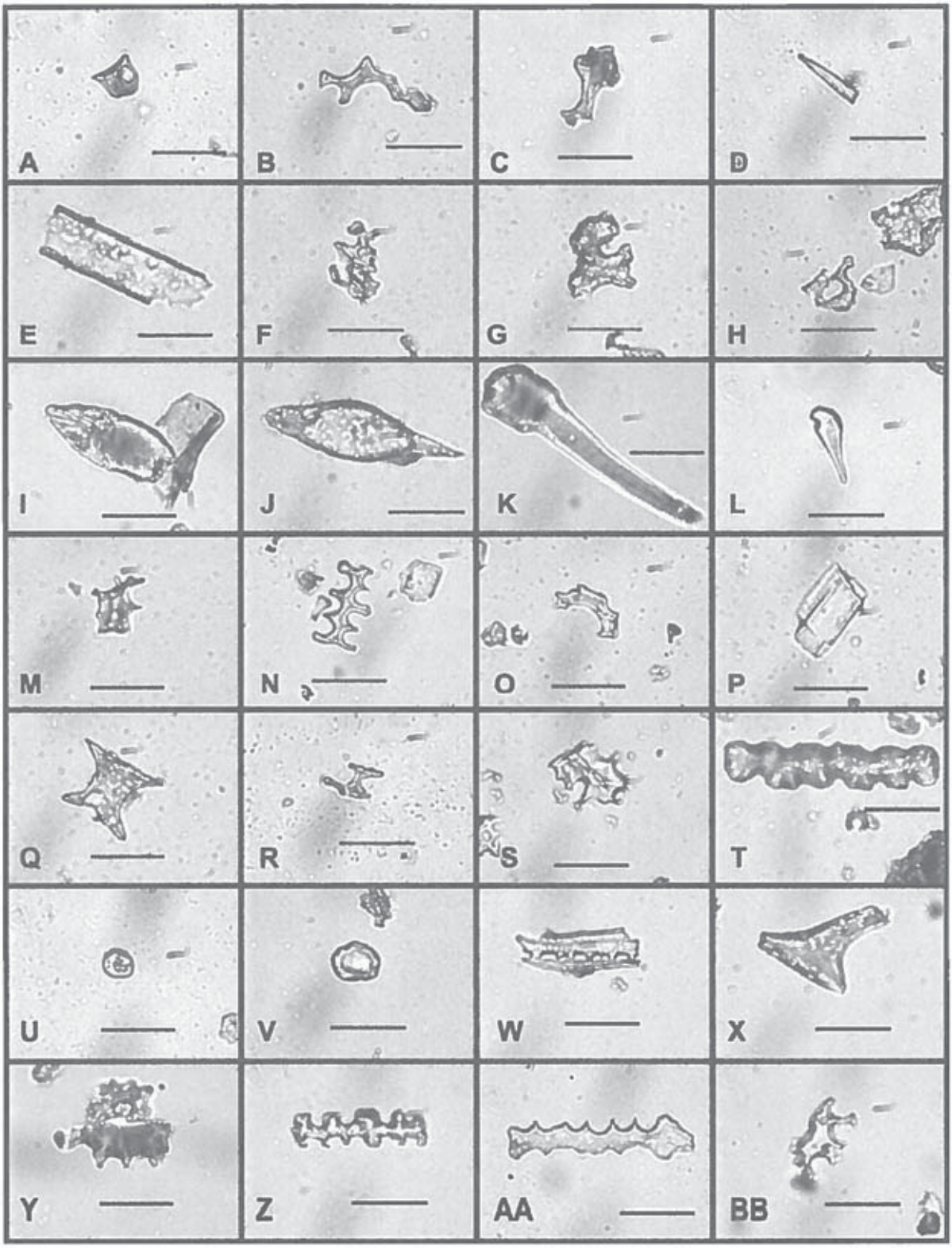

Figure D-14. Unidentified phytolith forms observed in these samples (41BL278). Images A-P = Sample 6; images Q-T = Sample 7; images U-X = Sample 8; and images Y-BB = Sample 9 (Sample identities in Table D-1). Bar scales are 25 microns.

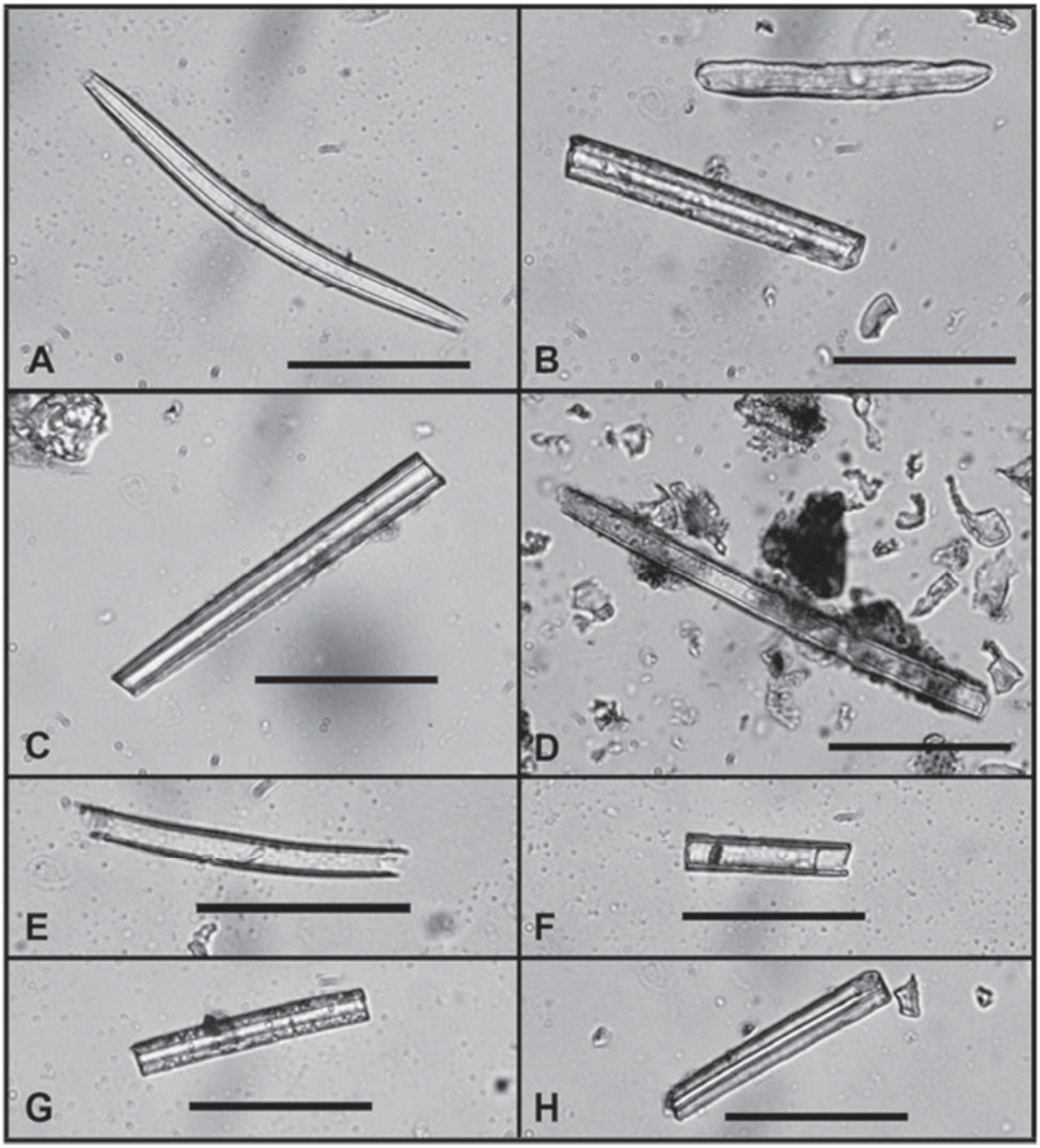

Figure D-15. Sample 6 spicules (41BL278). The upper right specimen in B is a phytolith of similar size to the spicule that is pictured (Sample identities in Table D-1). Bar scales are 50 microns.

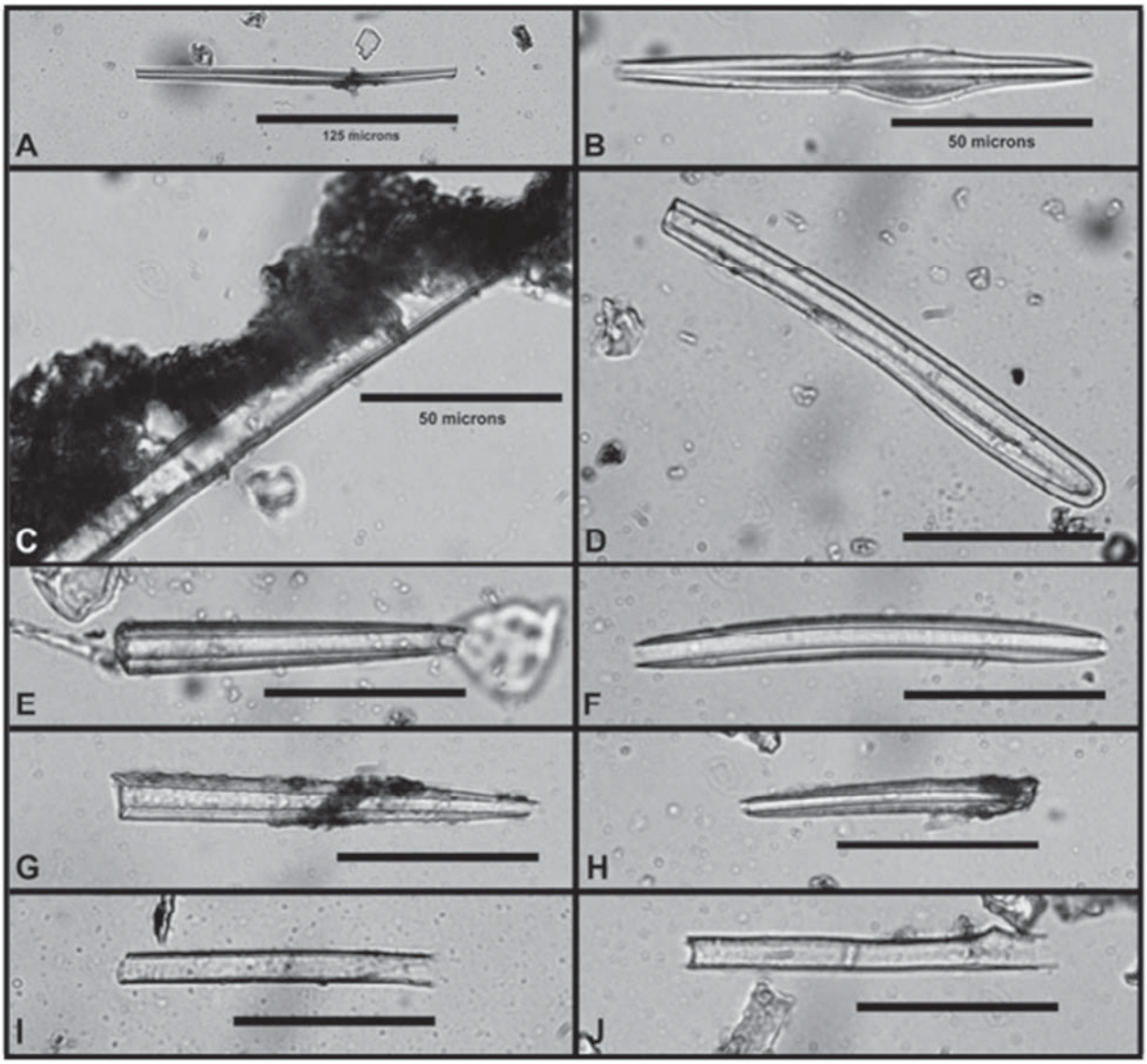

Figure D-16. Sample 7 and 8 spicules and similar particles (41BL278). A-E = Sample 7, F-J = Sample 8 (sample identities in Table D-1). All scale bars are 50 microns except for A which is 125 microns. Specimen J may not be a spicule; the identity of C and I is uncertain.

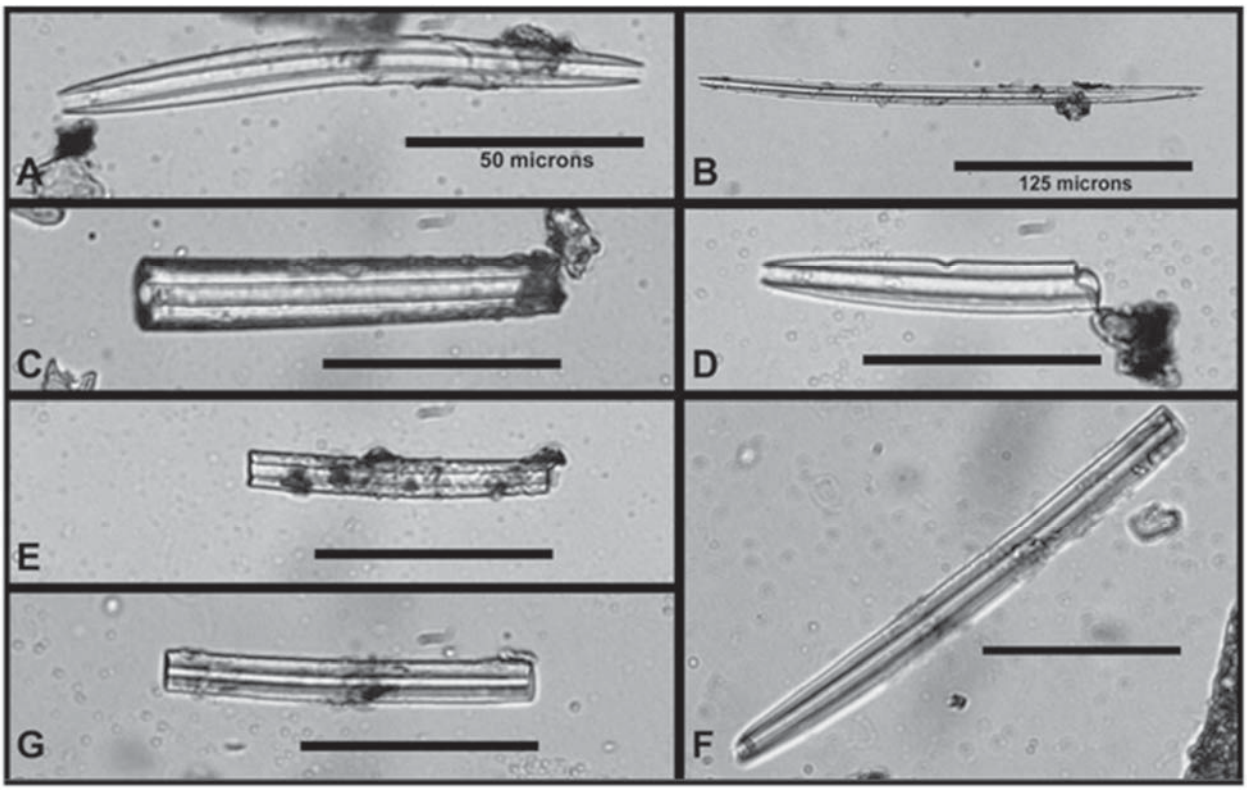

Figure D-17. Sample 9 spicules (41BL278) (Sample identities in Table D-1). Bar scale in B is 125 microns long [spicule photographed at 200x]; all other bar scales are 50 microns [spicules photographed at 500x].

strata so they were of variable thickness although the total profile was much thicker than at the Burnham Site. Work to date on 34WO69 has been summarized (Sudbury 2014b) and will be reported more fully in the future; that profile was sampled in 4 cm intervals to a depth of 1 meter (the 8 cm plow zone was pooled as one sample).

The samples analyzed from the Texas sites honed in on specific occupation features rather than complete soil profiles. A draft report documenting abundant snails and poor phytolith preservation at the Big Hole Site (41TV2161) has been prepared (Sudbury 2014a), and 41BL278--which also produced well preserved snails and poorly preserved phytoliths--is the topic of this current report. The samples from 41TV2161 were processed in sets of 12; the high carbonate content in the first two sets was neutralized in the 50 ml centrifuge tubes which turned out to be an extremely time consuming- strategy. As a result, the carbonates were not removed from the third 41TV2161 sample set and carbonate contamination of the phytolith isolates occurred (thus explaining the higher [0.18%] recovery for sample set 3 (Table D-3). To expedite laboratory processing, the biogenic silica isolates [instead of the whole silt fractions] from 41BL278 were acid treated to remove the carbonate contamination (Figure D-7) which resulted in a very low phytolith recovery of 0.05 weight percent in soil from 41BL278.

The previous carbonate issue encountered at 41TV2161 led to some literature investigation to understand the possible mechanism involved. The silica "bible" prepared by Iler (1979) was consulted, and a number of pertinent comments from that volume are documented in the Big Hole report (Sudbury 2014a). In summary the basic

Table D-3. ca. 5100 B.P. Occupational Level Soil Phytolith Concentrations.

Site Number	Phytoliths, wt. % in Soil	# Samples Averaged	Phytolith, wt. % Range in the Samples/Profile	Site Name/Comment [TX site's carbonate removal status]
41MS69	0.04 %	5	0.03 - 0.05% (n=5)	Spl 1-5, ~ same age (no CO_3)
41TV2161	0.09 %	12	0.04 - 0.14% (n=12)	Spl 1-12; soil column (no CO_3)
	0.10 %	12	0.08 - 0.16% (n=12)	Spl 13-24; samples (no CO_3)
	0.18 %	12	0.07 - 0.53% (n=12)	Spl 25-36; samples (CO_3 remains)
34WO69	2.09 %	3	0.80 - 4.11% (n=24)	Burnham 1 meter profile @ 4 cm
34NW132	1.10 %	1	0.46 - 4.60% (n=25)	Opossum Creek profile, by strata

issue was the caustic soil pH accelerated biogenic silica dissolution in the soil profile. This process can be accelerated by more water movement in the profile, and there are possible mechanisms whereby charcoal [and thus wood ash] and the action of other cations than calcium may also contribute to an accelerated dissolution rate.

The USDA soil type descriptions in the Official Series Descriptions (OSDs) were consulted for these four sites, and the significant soil information is summarized in Table D-4. The average carbonate equivalent range is the summary data provided on the USDA soil web site. The average carbonate range is the value given for the entire USDA tested-profile; the maximum range [which was only provided for 41BL278] lists the minimum and maximum values for the profile. The parent material and location relative to waterways is also noted. The modern day climate of the two Texas sites is similar, whereas both Oklahoma sites are slightly cooler. 34WO69 is significantly drier and 34NW132 is significantly wetter than the Texas sites. Both Texas sites clearly have calcic soils; the Oklahoma sites do not (although there was a trace of carbonate visible in the lower portions of the type profile cited as the type profile for St. Paul silt loam).

Table D-5 addresses the relative quality of snail and biogenic silica preservation at these same four sites. Clearly from this tabular summation, the basic calcic soils at the Texas sites provide excellent preservation for snails, but poor phytolith and biogenic silica preservation--with the apparent exception of siliceous sponge spicules. Conversely, no snails were recovered from the non-calcic Oklahoma sites, but the Oklahoma sites had exceptionally good phytolith and biogenic silica preservation--both in quantity and in apparent chemical weathering status. The sole trace microfossil difference between the two Oklahoma sites was in statospore abundance; this is interpreted as being due to 34NW132 being regularly wet, whereas prehistorically 34WO69 apparently dried out frequently which induces statospore formation (i.e., it had an intermittent water source, such as a spring, seep, or marshy area). The biogenic silica preservation improved in Table D-5 moving left to right as the soil carbonate content decreased. The point at which biogenic preservation deteriorates is bracketed in this data set, but the exact tipping point is not known--and may be somewhat variable due to specific localized conditions.

D.6 CONCLUSIONS

At 41BL278, there were abundant poorly preserved bulliform phytolith cells, and a relative dearth of short cell phytoliths recovered from the soil samples. The poor Poaceae phytolith preservation is felt to be due to dissolution caused by the calcic soil matrix. The phytolith evidence from

Table D-4. Site Soil Types and Carbonate Content (via USDA OSDs).

	41BL278	41TV2161	34WO69	34NW132
Soil Name (typical soil texture)	Venus[2] (loam)	Lewisville[3] (silty clay)	St. Paul[4] (silt loam)	Radley[5] (silt loam)
Soil Classification (Taxonomic Class)	Fine-loamy, mixed, superactive, thermic (Udic Calciustolls)	Fine-silty, mixed, active, thermic (Udic Calciustolls)	Fine-silty, mixed, superactive, thermic (Pachic Argiustolls)	Fine-silty, mixed, active, thermic (Fluventic Hapludolls)
Average CO_3 Equivalent	15-40%	20-40% [at 10-40"]	0-1% visible CO_3 below B [in BC & C]	0
CO_3 Equivalent Range	0-60%	# not provided	# not provided	0
Calcic Horizon (>15% CO_3)	14-60" (Bk or K)	16-62" (Bk)	none	none
Parent Material/ Base Residuum	Formed in loamy calcareous alluvial sediments of Pleistocene age	Formed in ancient loamy and calcareous sediments	Formed in silty Pleistocene alluvium over residuum of weathered Permian siltstone/sandstone	Formed in stratified silty alluvium
Flood Plain	Stream terraces and footslopes of valleys	Upland	Alluvial plain remnant	Nearly level flood plain
Comment	PZ also contains CO_3	PZ also contains CO_3	currently upland	frequently flooded
Soil Type Location Average Rainfall; Mean Temperature	28-40"; 62-69ºF	28-36"; 66ºF	21-28", 57-64ºF	38-47", 57-64ºF
Thornwaite Annual P-E index	44-64	44-66	32-44	64-82

41BL278 does show clear but non-species specific tree and fire data. No sedge phytoliths, statospores, or diatoms were observed in the sample biogenic isolates. The sponge spicules were well preserved--some fragments were weathered and/or abraded whereas many appeared to be pristine; some spicules were also complete, suggestive of enough available water available to pool and flow. Spicule presence in the feature is suggestive of water use in association with the feature. No reproductive spicules (i.e., gemmoscleres) were recovered.

A variety of snails were present in the sand fractions, as well as charcoal flecks, bone, burned shell (possibly snail), some lithic material, and other stone. Several sub- millimeter microflakes which looked like clear crystalline quartz that appeared to have platforms were observed (i.e., possible retouch flakes). Some slightly larger blocky apparent quartz debris was also noted in the sand.

The main component of the sand fraction--after quartz sand grains--appeared to be carbonate, some in chunks and shards, while other fragments appear to have definitely formed around roots when the roots were alive and/or recently decaying. The calcium concentration in the rhizosphere increases when the plants take up water; when CO_2 is given off in the root zone--either by bacteria or decaying roots--the calcium and the CO_2 form calcium carbonate (Bohn et al. 1979:131, 279-280; Birkeland 1984:139-140; Bouchardt 2002:714-715). Thus, the root traces in the carbonate seem to suggest that the carbonate in the rhizosphere was likely formed at or immediately after the time that the site was occupied. Samples 7 through 9 were collected from under limestone rocks in an effort to determine the environment at the time of occupation. Although some of the calcium and carbonate could have been derived from the soil parent material or the overlaying rocks, the general consensus in the literature is that the majority of

Table D-5. Biogenic Silica and Snail Frequency and Preservation at Study Sites.

	Site Number											
	41BL278			41TV2161			34WO69			34NW132		
	P[6]	A	Condition	P	A	Condition	P	A	Condition	P	A	Condition
Phytoliths	X		Poor, rare	X		Poor, rare	X		XL, abund	X		XL, abund
Diatoms		X	Absent	X		Poor, v rare	X		XL, abund	X		XL, abund
Sponge Spicules	X		XL	X		XL	X		XL, abund	X		XL, abund
Gemmoscleres		X	Absent	X		XL, scarce	X		XL, abund	X		XL, abund
Statospores		X	Absent	X		Poor, v rare	X		XL,v abund	X		XL, rare
Snails	X		XL, abund	X		XL, abund		X	Absent		X	Absent
Phytoliths, wt. %	0.05%			0.10%			2.09%			1.10%		
Soil Subgroup Great Group	Udic Calciustolls			Udic Calciustolls			Pachic Argiustolls			Fluventic Hapludolls		
Carbonate Content	0 - 60%			20-40%			0-1%			0		

2 https://soilseries.sc.egov.usda.gov/OSD_Docs/V/VENUS.html (accessed 8-3-14)

3 https://soilseries.sc.egov.usda.gov/OSD_Docs/L/LEWISVILLE.html (accessed 8-3-14)

4 https://soilseries.sc.egov.usda.gov/OSD_Docs/S/ST._PAUL.html (accessed 8-3-14)

5 https://soilseries.sc.egov.usda.gov/OSD_Docs/R/RADLEY.html (accessed 8-3-14)

6 "P" denotes presence; "A" denotes absence; "Condition" refers to weathering or preservation; "XL" denotes excellent condition; "v" as in v rare means "very" rare; "abund" means abundant. Gemmoscleres are reproductive sponge spicules

calcium present in soil is deposited at sites via aeolian deposition, or by dust settled by precipitation (Birkeland 1984:140-141; Goldberg and Macphail 2006:141-144). A significant carbonate content was also observed in the soil sample silt fractions.

The reason for the additional clay components present in the control soil (Sample 6) clay fraction (Figure D-8) is uncertain. One potential caution could be due to the samples collected. The other three samples were collected under rocks to evaluate the living surface, and thus were cut off from further aeolian deposition. The control sample remained exposed to normal soil aggradation, and thus may contain components that were added via post-occupation environmental deposition. The limestone rocks may have contributed to detrimental soil preservation of the three samples--but even the control sample sans rocks had poor phytolith preservation.

The abundance of well-preserved snails in high carbonate content soils presents an opportunity to harvest environmental information from that resource. In the future the sample preparation method could be optimized (i.e., gentler initial disaggregation) to enhance complete snail specimen recovery, and the recovered snails and/or snail images provided to a specialist for identification and environmental assessment.

In a very significant observation, it has been reported that diatoms--absent in the general site soil matrix at 41TV2161--were congregating at the root surfaces (presumably feeding) and were encapsulated and preserved in the carbonate nodules that formed in the rhizosphere (and also found preserved in carbonate nodules adjacent to bone) (Winsborough 2014). Thus, encapsulation by carbonate apparently shielded the diatoms from dissolution via basic pH coupled with migrating

soil pore water, and the carbonate may have maintained the local environment pH in the 8.2 to 8.5 range.

There are several potential issues that the archived soil carbonate-containing samples from 41BL278 could address.

1. Although soil phytoliths were not alive and actively feeding in the root zone, any phytoliths that were incidentally present in the carbonate encapsulated soil zone may be preserved in the carbonate nodules as the diatoms were. No sand origin carbonate nodules were dissolved to test this theory, but that does remain an option.
2. In the absence of good short cell phytolith samples, soil carbonate can be analyzed to determine the δ13C value of the soil, and thus provide an indication of the C3 vs. C4 plant community distribution that contributed the carbon at or very near the living surface (Schaetzl and Anderson 2005:648-649).
3. The carbonate can also be used to potentially determine the source of the carbonate at the site (i.e. aeolian vs. marine vs. pedogenic) (ibid.). This information can also be pertinent to the question addressed in point 2 in unraveling the ultimate source of the carbon in the carbonate. The sand fractions (and all soil fractions) have been curated at the J.S. Enterprise laboratory, and are available for any additional analysis if and when needed.

D.7 ACKNOWLEDGEMENTS

I gratefully acknowledge the contributions of Barbara Winsborough and Mike Quigg in the completion of this report.

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Archeological Testing of TxDOT Right-of-Way through Site 41BL278, Bell County, Texas

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TxDOT Scientific Services Contract Nos. 573XXSA006 and 57-3XXSA004

2015

Printed by:

Document Engine
Round Rock, Texas

Jointly published by:

Texas Department of Transportation
Environmental Affairs Division
Archeological Studies Program
Scott Pletka, Ph.D., Supervisor

Archeological Studies Program Report No. 171

and

TRC Environmental Corporation
TRC Technical Report Nos. 42568 (106760) and 211462
Austin, Texas

ISBN #978-1-935545-38-5

EXECUTIVE SUMMARY

In January 2004, Blanton & Associates, Inc., conducted an archeological survey of 8.5 hectares (21.1 acres) for a proposed roadway improvement and bridge replacement project (CSJ: 0396-04-059) along roughly 1,800 meters (m) of the Texas Department of Transportation (TxDOT) right-of-way where State Highway (SH) 317 crosses the Leon River in Bell County, Texas (Ringstaff 2004). That investigation consisted of three shovel tests, ten backhoe trenches, and five hand-excavated columns. Five backhoe trenches and the screened trench columns were excavated in the alluvial terrace on the southern side of the Leon River and encountered a single cultural component buried between 130 and 180 centimeters (cm) below surface (bs). Wood charcoal from burned rock Feature 1 yielded an accelerated mass spectrometer date of 2490 ± 50 B.P. As a result, boundaries of a previously documented prehistoric cultural resource site to the southeast, 41BL278 were extended northwestward to include the river terrace on the southern side of the Leon River where the bridge development, area of potential effect (APE) is proposed. Ringstaff (2004) recommended archeological testing/evaluation in the area of the APE at site 41BL278, if that part of the site could not be avoided by planned bridge expansion. The Texas Historical Commission concurred with this recommendation.

In June 2004, archeologists from the Cultural Resources Section of the Planning, Permitting, and Licensing Practice of TRC Environmental Corporation's (TRC's) Austin office conducted archeological testing/evaluation for a National Register of Historic Places (NRHP) and State Antiquities Landmark (SAL) eligibility assessment at parts of site 41BL278. This investigation was conducted under TxDOT Contract for Scientific Services No. 573XXSA006 and Texas Antiquities Permit No. 3446 issued to J. Michael Quigg. The assessment of the previously identified, deeply buried cultural component in the proposed APE and part of prehistoric site 41BL278 was accomplished by an electrical resistivity survey of 840 m^2 area, mechanical excavation of eight backhoe trenches (totaling nearly 34 linear meters), and 10 hand-excavated 1-by-1 meter units (totaling 4.1 m^3) off the sides of three backhoe trenches. The previously identified and targeted buried cultural component was determined to be vertically restricted between 130 and 180 cmbs and horizontally restricted to the levee-like deposit towards the northern edge of the terrace. The levee appears to have been deposited relatively rapidly.

This eligibility assessment yielded nine cultural features that included at least one circular, rock-ringed hearth and various types of burned rock discard piles and dumps, some with only burned rocks, some combined with other classes of cultural material. A limited, but diverse cultural assemblage of chipped and ground stone tools, burned rocks, mussel shells, faunal bone, and organic materials was directly associated with the nine features. These material remains indicate two short-term events, which potentially reflect food processing activities that occurred between 3100 and 3300 B.P. and reflecting one cultural component.

Based on the investigations, it is apparent that the part of site 41BL278 within TxDOT's proposed APE contains a well-defined, 50 cm thick cultural component between 130 and 180 cmbs. This component is characterized by probably two, horizontal lenses of cultural material restricted to a narrow time period (ca. 3100 to 3300 B.P.). Relatively sterile alluvium overlies this component, and rodent activity and other sources of natural disturbance have not significantly affected this deeply buried component.

Following the fieldwork the chipped and ground stone tools, lithic debitage, mussel shell, faunal

bone, charcoal, and other cultural materials were washed, sorted, and counted. A draft interim report of the fieldwork, findings, recommendations, and data recovery plan was prepared and submitted to TxDOT in 2004 (Quigg and Frederick 2004).

In November 2013, TxDOT issued Work Authorization 57-306SA004 to TRC under a Contract for Scientific Services (57-3XXSA004) to conduct limited technical analysis of samples, complete the draft and final technical reports, plus prepare the materials from the assessment phase for curation. Following completion of analyses, reporting and acceptance by TxDOT, these materials were permanently curated at the Center for Archaeological Studies (CAS) in San Marcos, Texas.

Multiple technical analyses included radiocarbon dating, starch grain, lipid residue, macrobotanical, high-powered use-wear, and phytolith studies. Poor preservation was the rule and definitely hindered, but did not prevent a greater understanding of the data. Poor preservation of the charcoal limited specific identification of most degraded samples, but at least two species, oak and cottonwood/willow were identified. Limited charcoal and other organic remains also restricted radiocarbon dating of the cultural activities. Tiny charcoal pieces provided five radiocarbon dates to aid in establishing the age of the targeted component to roughly 3200 B.P.

High-powered use-wear on at least three of the eight chipped stone tools analyzed documented cutting of wood products or other hard materials. Use-wear studies also revealed microscopic residues of wood, plant tissues, collagen, and feather fragments directly adhering to stone tools. Analyzed tools were also used to cut soft materials such as hides. Overall, the results reflect the occupants used chipped stone tools to conduct multiple processing tasks on diverse materials. Of considerable interest and quite rare is the feather fragments, which directly support the cutting of birds.

Starch analysis indicates at least two types of grasses, plus lily bulbs and other geophytes were collected, processed and cooked by burned rocks from multiple, small burned rock features. The presence of gelatinized starches on nearly 25 percent of the examined rocks indicates stone boiling likely occurred as a means of cooking starchy plant foods. Starch grain analysis has significantly broadened our understanding of exploited resources at this 3200 B.P. component, which would have otherwise gone undetected as the macrobotanical and vertebrate faunal remains are poorly preserved and very limited.

Phytolith analysis indicates very poor preservation of the important short cell phytoliths used to interpret grassland communities. Samples from three burned rock features yielded a variety of short cells (Pooids, Panicoids, and Chloridoids), but not sufficient for meaningful counts and interpretations. Also present were quantities of undiagnostic bulliforms, well-preserved burned tree phytoliths, and well-preserved freshwater sponge spicules, the latter are associated with water. Freshwater sponge spicule presence in features is indicative of water use in association with burned rocks. Due to improved phytolith processing techniques a variety of microscopic snail shells as well as charcoal flecks, bone fragments, burned shell (possibly snail), some lithic material, and other stone were present in the sand fractions.

Lipid residue analysis primarily yielded residues of medium fat content, with similar residues in plant and animal products. Residues from conifer products were on nearly every burned rock and likely testifies to the type of wood used to heat the rocks, here likely juniper or cypress.

Even though preservation was poor, the diverse technical analyses documented multiple plants, minimally grass and lilies/geophytes played a significant role, plus animals such as beaver and birds, combined with mussel shell meat provide a

broad spectrum forager pattern at this location. These diverse resources were cooked with burned rocks from the multiple features represented.

The investigated portion of site 41BL278 has yielded significant information and the remaining deposits in the APE has the potential to yield further important information pertinent to answering multiple research questions about local and regional prehistory. Therefore, the investigated area of 41BL278 in the APE is recommended as eligible for listing on the NRHP under Criterion D and for designation as an SAL. If this area cannot be avoided during the expansion of the bridge over the Leon River, TRC recommends that the part of site 41BL278 lying within the existing and proposed new right-of-way along this roadway at the Leon River crossing, specifically the cultural component buried between 130 and 180 cmbs, be targeted for a mitigation excavation program prior to any earth-disturbing activities.

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