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Experimental micropedology—A technique for investigating soil carbonate biogenesis along a desert-grassland-forest transect, New Mexico, USA

Microedafología experimental—Una técnica para investigar la biogénesis de carbonato en el suelo a lo largo de un transecto desierto-pradera-bosque, New Mexico, USA

Micropedologia experimental – Uma técnica para investigar a biogénese dos carbonatos no solo ao longo de um transecto deserto-pastagem-floresta, New Mexico, USA

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ABSTRACT

Manipulative experiments—characterized by comparing treatments to controls—are widespread in scientific investigations. This study uses *experimental micropedology* to investigate whether soil microbes precipitate carbonate if a liquid growth-medium is applied to soil *in situ*. This was undertaken using apparatuses designed to (1) obtain micromorphological images of biogenic carbonate on microscope slides, (2) to quantify carbonate formation in fiberglass cloths, and (3) to measure associated carbon-isotope fractionations. The apparatuses were buried and harvested at monthly intervals from December 2010 to June 2011. The study was conducted along an ecological transect in New Mexico, USA, at three sites: a low-elevation desert (C_3 shrubs), an intermediate-elevation steppe (C_4 grasses), and a high-elevation forest (C_3 conifers). In addition to comparing bioclimatic zones, the effect of parent material was also tested using paired limestone and igneous soils at each site. Soil samples in their natural state and inserted microscope slides were analyzed with binocular, petrographic, and scanning electron microscopy equipped with an x-ray microanalyser (EDS), and the fiberglass traps were analyzed with x-ray diffraction and a mass spectrometer for carbon concentrations and isotope ratios. Naturally occurring calcified microbes were found at each site in the form of calcified hyphae, needle fiber, and calcified root hairs, with the exception of the forest site on igneous parent material. Liquid growth medium induced microbial calcification regardless of whether the vegetation was desert shrubs, grassland, or forest, and regardless of whether the parent material was igneous or limestone. Thus, the ability of soil microorganisms to biomineralize carbonate when supplied with liquid growth medium *in situ* is a phenomenon that crosses biomes and is not limited to microbes endemic to either limestone or igneous parent material.

RESUMEN

Los experimentos manipulativos, caracterizados por comparar tratamientos con experiencias control, se utilizan ampliamente en las investigaciones científicas. Este estudio aplica la microedafología experimental para investigar si los microorganismos del suelo precipitan carbonato al aplicar in situ un medio de crecimiento líquido al suelo. Esto se llevó a cabo utilizando aparatos diseñados para (1) obtener imágenes micromorfológicas de carbonato biogénico sobre láminas delgadas, (2) cuantificar la formación de carbonato sobre telas de fibra de vidrio, y (3) medir fraccionamientos isotópicos de carbono asociados. Los aparatos fueron enterrados en el suelo y recogidos en intervalos mensuales desde diciembre de 2010 hasta junio de 2011. El estudio se realizó a lo largo de un transecto ecológico en New Mexico, USA, en tres localizaciones: un desierto de relieve suave (arbustos C_3), una pradera de relieve intermedio (pastos C_4) y un bosque de alto relieve (coníferas C_3). Además de comparar las distintas zonas bioclimáticas también se analizó el efecto del material parental estudiando suelos sobre rocas carbonatadas

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e ígneas en cada lugar. Las láminas delgadas se analizaron con microscopía binocular, petrográfica y electrónica de barrido equipada con un mincroanalizador de rayos X (EDS), y las trampas de fibra de vidrio se analizaron mediante difracción de rayos X y un espectrómetro de masas para obtener las concentraciones de carbono y las razones isotópicas. En cada localización se encontraron microorganismos calcificados existentes de forma natural en forma de micelios calcificados filamentosos y pelos de raíces calcificados, con la excepción del lugar sobre material ígneo en la zona de bosque. El medio de crecimiento líquido indujo la calcificación microbiana independientemente del tipo de vegetación o de material parental. Así, la habilidad de los microorganismos del suelo para biomineralizar carbonato mediante la aportación in situ de un medio de crecimiento líquido es un fenómeno transversal independiente del bioma y que no está limitado a microorganismos endémicos de materiales calizos o ígneos.

RESUMO

Os ensaios de manipulação — caracterizados pela comparação dos tratamentos com as testemunhas (controlos) — estão largamente difundidos em investigação científica. Este estudo utiliza a micro pedologia experimental para investigar se os microrganismos do solo precipitam carbonato quando se aplica um meio de crescimento líquido ao solo in situ. Este trabalho foi levado a cabo utilizando dispositivos projetados para (1) obter imagens micromorfológicas de carbonato biogénico em lâminas finas, (2) quantificar a formação de carbonato em tecidos de fibra de vidro, e (3) medir o fracionamento isotópico de carbono associado. Os dispositivos foram enterrados no solo e recolhidos com intervalos mensais de dezembro de 2010 até junho de 2011. O estudo foi realizado ao longo de um transecto ecológico no Novo México, EUA, em três locais: um deserto de relevo suave (arbustos C3), uma planície de relevo intermédio (gramíneas C4) e uma floresta de alto relevo (coníferas C3). Além de comparar áreas bioclimáticas foi também analisado o efeito do material parental estudando em cada local solos localizados sobre rochas carbonatadas e rochas magmáticas. As lâminas finas foram analisadas por microscopia binocular e petrográfica e microscopia eletrónica de varrimento equipada com um micro analisador de raios- X (EDS), e os tecidos de fibra de vidro foram analisados por difração de raios X e espectrometria de massa para obter concentrações de carbono e relações isotópicas. Em cada local foram encontrados microrganismos calcificados formados naturalmente em forma de micélios calcificados filamentosos e pelos de raízes calcificados, com exceção do local sobre material ígneo na zona de floresta. O meio de crescimento líquido induziu à calcificação microbiana, independentemente do tipo de vegetação ou material parental. Assim, a capacidade dos microrganismos do solo para biomineralizar carbonato mediante o fornecimento no local de um meio de crescimento líquido é um fenómeno transversal independente do bioma e não está limitado aos microrganismos endémicos de materiais calcários ou ígneos.

KEYWORDS
Pedogenic carbonates, biomineralization, needle-fiber calcite, soil micromorphology

PALABRAS CLAVE
Carbonatos edáficos, biomineralización, calcita filamentosa, micromorfología de suelos

PALAVRAS-CHAVE
Carbonatos pedológicos, biomineralização, calcite filamentosa, micromorfologia do solos

1. Introduction

Micropedology, like pedology, studies soil as a natural phenomenon, taking into account its composition, distribution and method of formation (FitzPatrick 1972, 1993). Micropedology and pedology differ from other disciplines of soil science because they work with undisturbed soil in its natural environment. Still, soil samples must be taken to the lab and "a soil sample really is not soil" (Kubiëna 1970). Moreover, fabric analysis using petrographic methods, like profile analysis using field methods, is a descriptive science (Brewer 1964; Bullock et al. 1985; Stoops 2003). This study takes a manipulative-experimental approach used in many agricultural, forestry, and environmental studies by comparing treatments with controls. Rather than taking samples to the lab, it brings the microscope slides to the natural soil in the field. It adds liquid growth medium to soil microhabitats to stimulate indigenous microbial populations. The focus of this study (i.e., the response variable of this manipulative experiment) is soil carbonate.

Soil carbonate is an important soil component because it serves as an indicator of landscape stability and paleoclimate, it affects nutrient availability, and it has implications for carbon sequestration (Gile et al. 1966; Cerling 1984; Monger et al. 2011). Soil carbonate has been traditionally viewed as a soil mineral, which, of course, it is. The problem with this viewpoint, however, is that it considers soil $CaCO_3$ to be geological, not biological. That is, carbonate formation and dissolution, like other soil minerals, are governed by abiotic physicochemical processes (Marion et al. 1985; Marion et al. 2008; Breecker et al. 2009; Hirmas et al. 2010; Schlesinger et al. 2009). Without invoking biomineralization processes, many authors have traditionally, and successfully, modeled the precipitation and dissolution of calcium carbonate in soil using the classical equation below:

$$Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + CO_2 + H_2O$$

Soil carbonate does, however, have a long history of investigations that indicates a biological origin, at least in part. In an early study on the topic, Krumbein (1968) identified bacteria in a calcrete from Israel that could precipitate calcite when cultured in solid media. Boquet et al. (1973) found that many strains of bacteria, including *Salmonella* spp., *Bacillus pumilus*, and *Pseudomonas aeruginosa*, could form calcite crystals in 1 to 20 days on a medium containing calcium acetate. Biomineralization of calcretes and needle fiber calcite as a biologically-produced mineral have been recognized since the 1990s (Verrecchia 1990; Verrecchia and Verrecchia 1994). Field studies have provided evidence for organic formation of calcretes (Goudie 1996), while micromorphology studies have revealed calcified hyphae in petrocalcic horizons of limestone soils in central Texas (Wilding et al. 1997). Soil microorganisms were also found to be involved in calcite precipitation in a typical desert soil near Las Cruces, New Mexico where fossilized remains of calcified fungal hyphae are abundant (Monger et al. 1991). In lab experiments, soil bacteria and fungi precipitated calcite when cultured on a Ca-rich medium. In an experiment where soil columns were irrigated with Ca-rich solutions, calcite formed in soils containing soil microorganisms, but no calcite formed in sterile soils. Thus, calcic and petrocalcic horizons are not simply the result of inorganic precipitation of calcite.

More recently, Burford et al. (2006) investigated the roles of fungi in the transformation of limestone and provided direct experimental evidence of fungal-mediated carbonate precipitation when cultured under low nutrient conditions on a solid limestone substrate. Their results revealed calcite biomineralization by two strains of fungi grown in buffered neutral to alkaline conditions, saturated with respect to carbonate.

Verrecchia et al. (2006) emphasize a key role of fungi in the oxalate-carbonate pathway and its importance as a major global carbon sink. In a well-buffered neutral to alkaline environment containing sufficient amounts of Ca^{2+}, Mg^{2+}, some of the CO_2 will be transformed into carbonate, which will precipitate with the appropriate cations. Thus, precipitation of $CaCO_3$ on fungal hyphae could be the result of fungal metabolic processes, and also a consequence of bacterial oxalate degradation (Verrecchia et al. 1990; 2006). Similarly, Cailleau et al. (2011) found that calcite biomineralization in trees is related to the oxalate oxidation in soil. The consequence of this oxidation is the presence of carbonate ions in the soil solution pumped through the roots, leading to preferential mineralization of the roots and the trunk base.

Worldwide, several SEM studies of calcareous terrestrial deposits have shown the importance of soil microorganisms in biomineralization of carbonate. Such studies have revealed fossilized communities of soil microorganisms in samples from the Florida Keys (Kahle 1977), the western Mediterranean area (Klappa 1979), southern Australia (Phillips et al. 1987), and the British West Indies (Jones 1988). In the southern High Plains of Texas, SEM and thin-section analyses revealed calcified filaments and *Microcodium* structures in petrocalcic horizons (Chitale 1986). These calcified filaments are similar in size and shape to filaments that Phillips et al. (1987) and Jones (1988) attributed to calcified fungal hyphae. Monger and Adams (1996) observed the fossilized remains of calcified

fungal hyphae and *Microcodium* structures in petrocalcic horizons of Nevada. Moreover, because pedogenic carbonates provide important evidence about paleoclimate and because organisms generally prefer lighter isotopes, it remains to be determined if microbial biomineralization causes fractionation of carbon isotopes (Cerling 1984; Quade et al. 1995; Monger et al. 2009).

Using experimental micropedology, this study addresses questions concerned with how widespread carbonate biomineralization is in different, but adjacent, bioclimatic zones and parent materials. It focuses on the possibility that indigenous microorganisms can be manipulated to generate pedogenic carbonate *in situ*. If so, does the type of ecosystem make a difference? Does parent material make a difference? Do microbes in these different ecosystems fractionate carbon isotopes differently? To answer these questions the following micropedology field experiment was conducted.

2. Materials and Methods

2.1. Bioclimatic setting

Sample sites along an elevation transect in southern New Mexico were chosen that are located in a desert shrubland in the Chihuahuan Desert, a grassland in a neighboring semiarid steppe, and a forest in the adjacent sub-humid mountains (**Figure 1**). The desert shrubs use the C_3 photosynthetic pathway, the grasses use the C_4 pathway, and the conifers use the C_3 pathway, and are thus distinguishable by their isotopic signatures (Cerling 1984). The desert site is at an elevation of 1318 m, has a mean annual max temperature of 26 °C, and a mean annual rainfall of 200 mm. The grassland site is at an elevation of 1681 m, has a mean annual max temperature of 23 °C, and a mean annual rainfall of 300 mm. The forest site is at an elevation of 2300 m, has a mean annual max temperature of 17 °C, and a mean annual rainfall of 560 mm. Greater than 50 percent of the precipitation comes in the summer months as monsoonal rains in

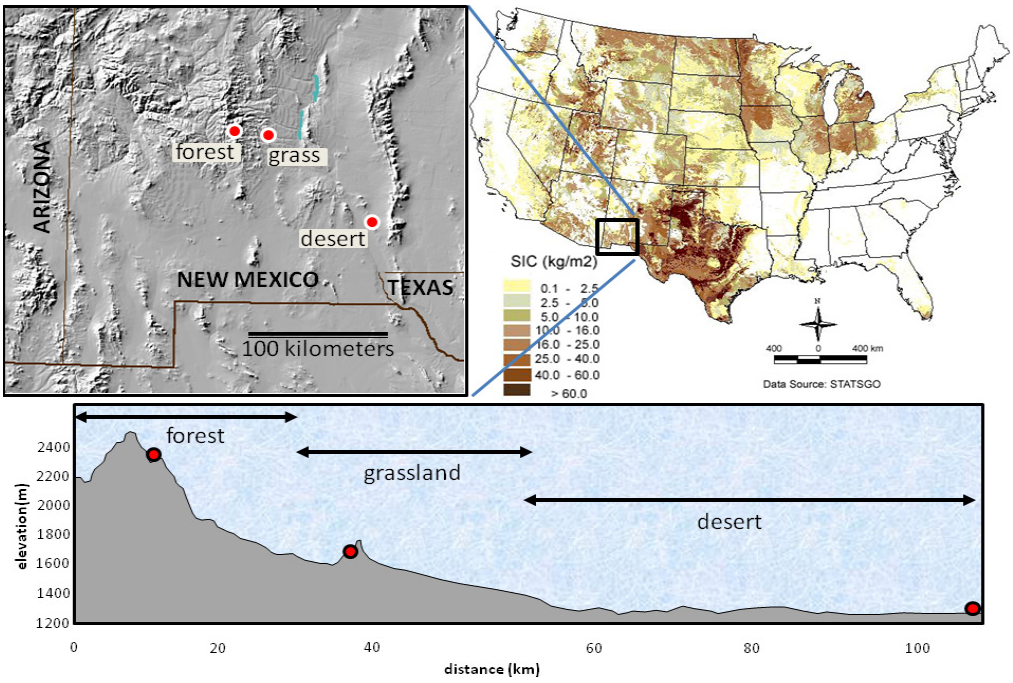

Figure 1. Location of study sites and comparison of their relative elevations. Mean annual temperatures and precipitation are given in the text. The general location of the study area is shown in reference to the distribution of soil inorganic carbon in the United States (Guo et al. 2006).

July, August, and September (Gile et al. 1981). The soil moisture and temperature regimes, as defined in Soil Taxonomy (Soil Survey Staff 1999), range from aridic thermic at the desert site, ustic-aridic thermic at the grassland site, and ustic mesic at the forest site. An extended drought occurred during the period in which this experiment was conducted, so the study area received little, if any, rainfall. For example, only 5 mm was recorded at the desert site for the duration of this study (Dec 2010 to June 2011), which was carried out based on the first-author's sabbatical visit.

Vegetation at the desert site is dominated by creosotebush (*Larrea tridentata)* and ratany (*Krameria parvifolia*), with scattered grasses (*Aristida and Bouteloua*) and cacti (*Opuntia*). The steppe grassland site is dominated by black grama (*Bouteloua eriopoda*), while the forest site is dominated by conifers, mainly ponderosa pine (*Pinus ponderosa*), alligator juniper (*Juniperus deppeana*), and pinyon pine (*Pinus edulis*), with scattered gamble oak (*Quercus gambelii*) (Dick-Peddie 1993).

Each of the three study sites were located in areas where soils formed in igneous parent materials were adjacent to soils formed in limestone parent materials. At each site the studied soils were within 200 meters of each other to minimize differences in morphology and genesis of soils that can occur within short distances (Wilding et al. 2001). Soil pits and rock types were described, sampled, and classified following the terminology described by Schoeneberger et al. (2012) (**Table 1**).

Profiles of soils at the desert sites are shown in **Figure 2**. Both soils formed in alluvium. The igneous alluvium is from Tertiary rhyolite of the Soledad formation in the Organ Mountains to the east (Seager et al. 1987). The limestone alluvium is from the Permian Hueco formation from Tortugas Mountain to the south. The primary difference between the morphology of the desert soils is an argillic horizon and petrocalcic horizon in the igneous soil, and calcic horizon in the limestone soil. The igneous soil is associated with a geomorphic surface that is older than the adjacent limestone soil, which

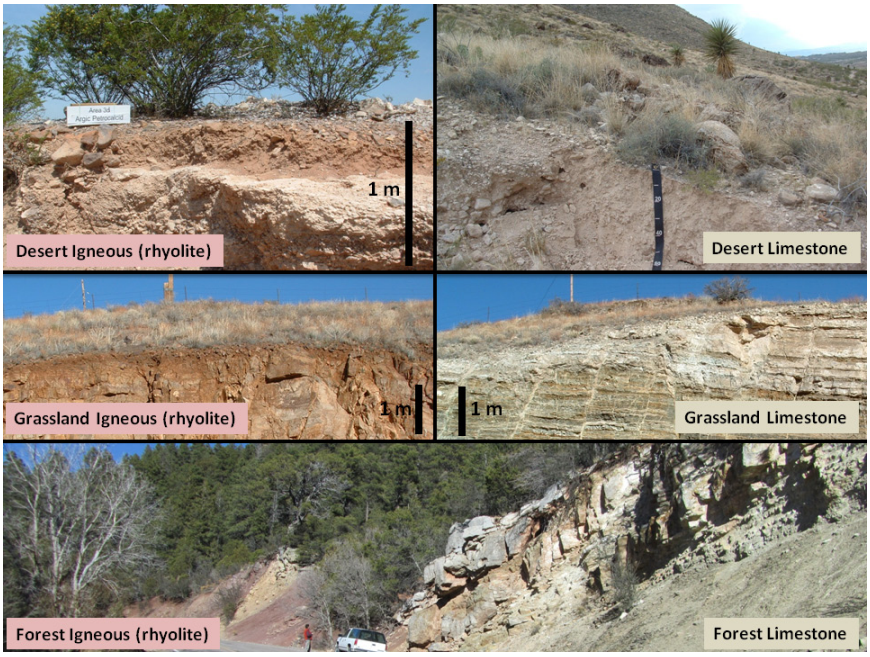

Figure 2. Photographs of soils profiles in the desert (top), grassland (middle), and forest (bottom). Each pair is with 200 meters of each other so that differences in the soil forming factors could be minimized.

Table 1. Morphological, geomorphic, and vegetative characteristics of the study sites

Site/Horizon	Depth cm	Textural Class	Carbonate Stages	Carbonate Forms†	Landform/ Material‡
Desert Igneous, Argic Petrocalcid					Fan-Piedmont/ Alluvium
Ak	0-5	sandy loam	Stage I	filaments	
Btk	6-28	sandy clay loam	Stage I	filaments	
Bkkm	28-43	sandy loam	Stage IV	laminar/plugged	
Bk	43-64	sandy loam	Stage III	plugged horizon	
Ck	64-79	loam	Stage I	pebble coatings	
Desert Limestone, Typic Haplocalcid					Fan-Piedmont/ Alluvium
Ak	0-10	sandy loam	Stage I	filaments	
Bkk1	10-30	sandy loam	Stage III	plugged horizon	
Bkk2	30-100	sandy loam	Stage III	plugged horizon	
Grassland Igneous, Ustic Haplargid					Hill Backslope/ Residuum
A	0-8	loam	–	–	
Btk	8-20	clay loam	Stage I	pebble coatings	
Rtk	20-300	-	Stage I	joint fillings	
Grassland Limestone, Ustic Haplocalcid					Hill Backslope/ Residuum
A	0-10	silt loam	–	none	
Bk	10-20	silt loam	Stage II	coatings/masses	
Crk	20-50	-	Stage II	coatings/masses	
Rk	50-300	-	Stage II	joint fillings	
Forest Igneous, Typic Haplustalf					Mountain Backslope/ Colluvium
A	0-20	sandy loam	–	–	
Bt	20-50	clay loam	–	–	
Cr	50-150	-	–	–	
R	150-300	-	–	–	
Forest Limestone, Typic Calciustept					Mountain Backslope/ Colluvium
A	0-20	silt loam	–	–	
Bk	20-60	silt loam	Stage I	pebble coatings	
Crk	60-200	-	Stage I	ped coatings	
Rk	200-300	-	Stage I	joint fillings	

† Carbonate stages and forms from Gile et al. 1966 and Schoeneberger et al. 2012.
‡ Landforms from Peterson 1981.

explains why one has a petrocalcic horizon (the older) and the other does not (Gile et al. 1981). The igneous soil is classified as an Argic Petrocalcid and the limestone soil as a Typic Haplocalcid (Gile et al. 2003).

Soils at the grassland site are formed in bedrock residuum on backslopes of hills. The igneous and limestone bedrock are juxtaposed along a fault. The igneous rock is Tertiary rhyolite and the limestone is Cambrian in age (New Mexico Bureau of Geology and Mineral Resources 2003). The A horizons in the grassland sites are thicker and more distinct than A horizons at the desert sites. Another difference is less pedogenic carbonate in the igneous soil. The igneous soil at the grassland site is an Ustic Haplargid while the neighboring limestone soil is an Ustic Haplocalcid (**Figure 2**).

The forest soils also have Tertiary volcanic rocks juxtaposed with limestone along a fault; however, these limestones are Pennsylvanian to Mississippian in age. No pedogenic carbonate is apparent in the igneous soil forest soil, unlike its limestone neighbor (**Table 1**). The pH values of all horizons in the desert and grassland soils range from about 7.8 to 8.3 (Gile et al. 1981). Forest soils in New Mexico at elevations of 2300 meters typically have pH values ranging from 7.2 to 7.8 (Folks 1975). The igneous soil at the forest site formed in colluvium and is classified as a Typic Haplustalf on a mountain blackslope. The limestone soil is a Typic Calciustept formed in colluium on an adjacent mountain backslope.

2.2. Experimental design

This study was designed to take growth media to the field soil in order to observe if carbonate biomineralization occurs *in situ*, and whether ecosystems and parent materials are factors that impact carbonate formation. To achieve this, we designed an apparatus that could be buried in soils, deliver a liquid growth medium, and then be retrieved to observe biomineralization. The apparatus consists of a sterile vial into which a liquid medium was poured, two nylon wicks containing fiberglass cloth sown between the wicks, and an attached microscope slide (**Figure 3**). This design combined micromorphology with the ability to measure the amount of biogenic carbonate generated in the fiberglass cloth, which could be measured by dry combustion of the total carbon minus the organic carbon. Using a mass spectrometer attached to an elemental analyzer, carbon isotopes could also be measured. Moreover, the properties of biomineralization could be observed by attaching a microscope slide to the wicks.

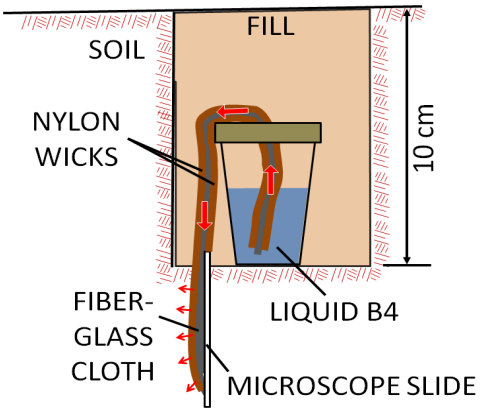

Figure 3. Apparatus used to deliver liquid growth medium to soil in order to observe *in situ* carbonate biomineralization on the microscope slide with a petrographic microscope. The fiberglass cloth enclosed between nylon wicks provides a means to measure carbonate and its $\delta^{13}C$ values with an elemental analyzer attached to a mass spectrometer.

The apparatus was inserted 10 cm into the soil with the glass slide extending to 15 cm (**Figure 3**), which was a depth that ensured the apparatus was protected from temperature extremes, animals, and erosion. This depth resulted in the slide being in the upper Bk or Bt horizons. Deeper depths would have made it more difficult to retrieve and replace the apparatuses. The growth medium was the "B4" medium which consists of 2.5 g calcium acetate, 4.0 g yeast extract, 15.0 g agar, and 10.0 g glucose per 1000 ml of distilled water, with the pH adjusted to 8.0 with NaOH (Boquet et al. 1973). We used a liquid form which consisted of the same ingredients but without the agar. This growth medium was autoclaved and taken to the field in a sealed sterile container. Likewise, to minimize the possibility of contamination, all apparatuses were sealed in plastic bags from the time of their construction in the lab until they were opened in the field. Growth medium was poured into each apparatus just prior to inserting them in the 10 cm hole. The liquid medium then moved through the wick and into the dry soil.

In December 2010, six apparatuses were installed at each site. These are referred to as "cumulative" samples because one of the six was harvested one month after installation, a second two months after installation, a third three months afterwards, and so forth until all six apparatuses were harvested by June. A second set of apparatuses were installed monthly and are referred to as "increment" samples. In this case, one apparatus was placed in the soil in January and harvested in February when another apparatus was put in the ground and harvested in March, and so forth until the last apparatus was harvested in June.

To serve as controls, apparatuses filled with water instead of B4 medium were buried and harvested in monthly intervals. Also to serve as controls, bare microscope slides were buried and harvested at monthly intervals. For each site, a total of 20 slides had been inserted into the soil and harvested by the end of the experiment. No statistical significance was applied to differences found among the sites because of the low sample size.

2.3. Biomineralization features

Features of biomineralization that occur naturally in soil, such as calcified roots and mycelia, were investigated in the field using a hand lens. At the microscopic scale, these features were viewed in the lab with a binocular microscope and with a scanning electron microscope (SEM) with an energy dispersive x-ray microanalysis EDS system (Hitachi, S-3400N Type II, Pleasanton, CA).

Biomineralization on the treated and control microscope slides were observed for each slide with a binocular microscope followed by observation in both plane- and crossed-polarized light using a petrographic microscope at 40x and 100x magnification. Carbonate was identified by its birefringence at a thickness from 4 µm to about 30 µm. Carbonate was also identified by spot-checking with applications of 1M HCl and observing effervescence in combination with elemental analysis with the scanning electron microscope. Based on these observations, a table documenting "presence or absence" was created. Carbonate formation in the fiberglass and on roots and hyphae was checked with x-ray diffraction (Rigaku MiniFlex). The fiberglass samples were attached to XRD sample holders using drops of deionized water that, after the water dried, held the samples firmly enough to the holders that they could be analyzed with the instrument.

The amount of carbonate produced in the fiberglass cloth as inorganic carbon, and its $\delta^{13}C$ values, were measured using an elemental analyzer attached to a continuous-flow isotope ratio mass spectrometer (Micromass, Waters, Inc). This was determined by weighing the fiberglass samples with a microbalance and placing them in tin capsules that were inserted into the autosampler on the elemental analyzer. In order to differentiate organic carbon from inorganic carbon, splits of each sample were made. One split was analyzed for total carbon. The other split was analyzed for organic carbon after immersing the fiberglass in a solution of 1M HCl until effervescence ceased. Inorganic carbon was calculated by subtracting organic carbon from total carbon.

Isotopic determinations were made on several natural vegetation, soil organic, and soil inorganic carbon samples (**Table 2**) using the following process (Weems and Monger, 2012). The fraction of carbon in the soil organic matter derived from C_4 plants (F_{C4}) was estimated by the equation (Boutton et al. 1999):

$$F_{C4} = \frac{\delta_{SAMPLE} - \delta_{C3}}{\delta_{C4} - \delta_{C3}} \quad (1)$$

Table 2. Isotopic values for vegetation and soil samples at the study sites

Site/Sample	Material	$\delta^{13}C$	% C4‡
Desert Igneous			
Larrea tridentata	shrub/leaves and stems	-25 ‰	
Btk horizon (6-28 cm)	soil organic matter	-21 ‰	34
Btk horizon (6-28 cm)	carbonate filaments	-12 ‰	0
Bkkm horizon(28-43 cm)	organics in petrocalcic	-16 ‰	82
Bkkm horizon(28-43 cm)	carbonate in petrocalcic	-3 ‰	64
Desert Limestone			
Acacia greggii	shrub/leaves and stems	-26 ‰	
Aristida divaricata	grass/leaves and stems	-14 ‰	
Bkk1 (10-30 cm)	soil organic matter	-16 ‰	82
Bkk1 (10-30 cm)	carbonate in calcic horizon	-7 ‰	36
Grassland Igneous			
Bouteloua eriopoda	grass/leaves and stems	-15 ‰	
A horizon (0-8 cm)	soil organic matter	-15 ‰	91
A horizon (0-8 cm)	disseminated carbonate	-5 ‰	50
Btk horizon (8-20 cm)	soil organic matter	-14 ‰	100
Btk horizon (8-20 cm)	carbonate coatings	-1 ‰	79
Forest Igneous			
Pinus edulis	needles and stems	-26 ‰	
A horizon (0-20 cm)	soil organic matter	-22 ‰	27
Forest Limestone			
A horizon (0-20 cm)	soil organic matter	-25 ‰	0
A horizon (0-20 cm)	disseminated carbonate	-13 ‰	0
Crk horizon (60-200 cm)	soil organic matter	-23 ‰	18
Crk horizon (60-200 cm)	carbonate coatings	-9 ‰	21

‡Theoretical percent of C4 vegetation based on equations 1 and 2.

where δ_{SAMPLE} is the $\delta^{13}C$ of the bulk soil sample, δ_{C3} is the average $\delta^{13}C$ value of the C_3 components, δ_{C4} is the average $\delta^{13}C$ value of the C_4 components. End-member values of -25‰ were used for C_3 plants and -14‰ for C_4 plants (Boutton et al. 1999). The C_4 fraction based on pedogenic carbonate was estimated by the equation:

$$F_{C4} = \frac{\delta_{SAMPLEpedcarb} - \delta_{C3\ pedcarb}}{\delta_{C4\ pedcarb} - \delta_{C3\ pedcarb}} \quad (2)$$

where $\delta_{SAMPLEped\ carb}$ is the $\delta^{13}C$ of the carbonate in the soil sample, $\delta_{C3ped\ carb}$ is the average $\delta^{13}C$ value derived from the C_3 components, $\delta_{C4ped\ carb}$ is the average $\delta^{13}C$ value derived from the C_4 components. End-member values of -12‰ were used for C_3 plants and +2‰ for C_4 plants (Quade et al. 1995). The amount of fractionation caused by microbial precipitation of carbonate was to be determined against the $\delta^{13}C$ value of the B4 medium measured to be -14‰.

3. Results and Discussion

3.1. Natural biomineralization features

Natural biomineralization in the form of calcified fine roots and mycelia could be observed in all soil profiles with a 10x hand lens, except for the igneous forest soil. These biological forms were most easily observed on the surfaces of coarse fragments. At the microscopic scale, various combinations of calcified root hairs, fungal hyphae, and needle fiber calcite were identified in all soil profiles with both the binocular scope and SEM (**Figure 4**), except for the igneous soil at the forest site. A chemical spectrum was made of the needle fiber at the grassland site (**Figure 5**) and indicates calcite. X-ray diffraction was used to analyze the calcified roots and fungal hyphae at the forest limestone site to check for Ca oxalate, but only calcite was revealed (**Figure 6**).

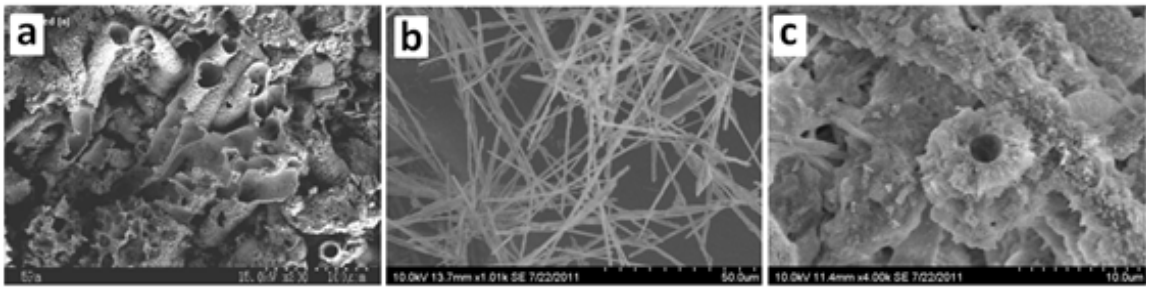

Figure 4. Examples of natural biomineralized forms of carbonate. **(a)** Calcified root hairs at the desert site with limestone parent material. **(b)** Needle-fiber at the grassland site. **(c)** Calcified fungal hyphae at the forest limestone site.

3.2. Biomineralization on microscope slides

Microscopic analysis of buried microscope slides attached to the nylon wicks revealed the following. Calcified fungal hyphae were the only form of biomineralization discernible with the binocular and petrographic microscopes. If bacterial biomineralization occurred, it was too small to document with light microscopy. Fungal calcification occurred as linear crystalline segments along the hyphae (**Figure 7**). The hyphae were most abundant along the sides and back of the microscope slides. The fiberglass cloth was analyzed by x-ray diffraction to test for calcite, but no calcite peaks were discernible within the broad peak produced by the amorphous glass. Neither was calcium oxalate detected by XRD. The amount of carbonate generated in the fiberglass was too small to obtain $^{13}C/^{12}C$ ratios with the mass spectrometer.

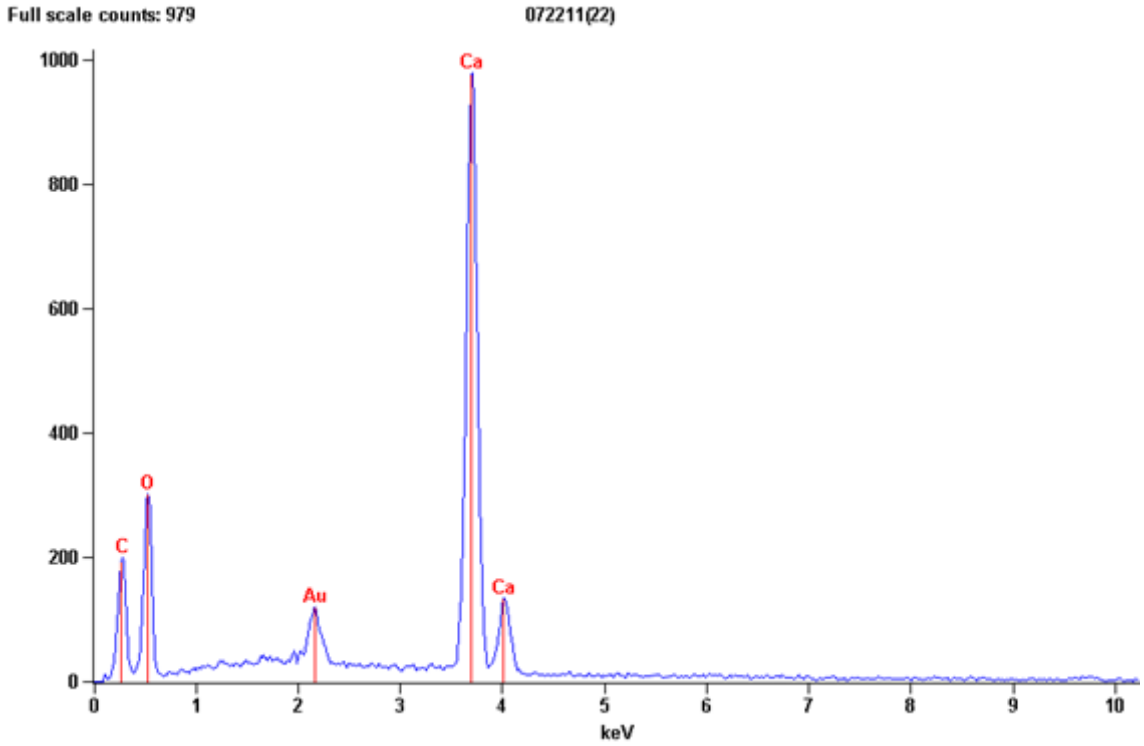

Figure 5. Chemical spectrum of needle fiber at the grassland site using SEM-EDS showing peaks of calcium (Ca), oxygen (O), and carbon (C) consistent with the molar concentrations of calcite. The gold (Au) peak is due to the sample coating.

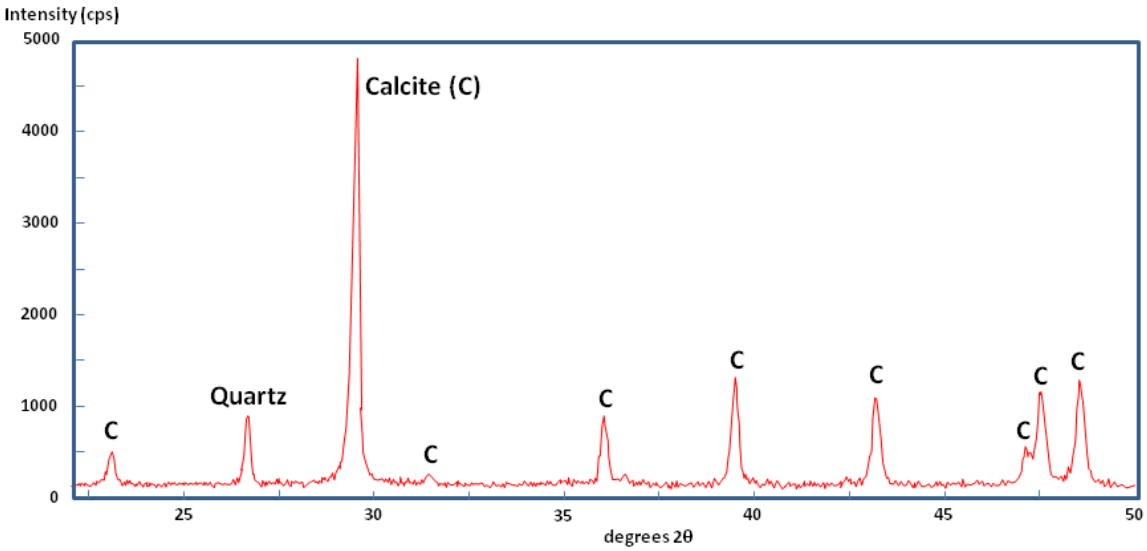

Figure 6. X-ray diffractogram of calcified roots and fungal hyphae coatings at the limestone forest site shown above in Figure 4C.

Calcium oxalate whewellite ($CaC_2O_4 \cdot H_2O$) and weddellite ($CaC_2O_4 \cdot 2H_2O$) are organic minerals occurring in various biological and geological environments. They are reported to be important for carbonate formation by microorganisms (Verrecchia et al. 1990, 2006) and for trees (Cailleau et al. 2011). X-ray diffraction of our samples, however, did not reveal precursor calcium oxalate crystals. Previous XRD analyses of soils in the study area also have not revealed calcium oxalate (Kraimer and Monger 2005). To date, calcite is the only pedogenic carbonate mineral associated with microbial biomineralization found in the soil environment of the study ares. However, calcium oxalate is commonly observed within plant roots in the study area. These crystals have similar appearances to those shown in Durand et al. (2010, Figure 21).

The density of hyphae and their calcification was very heterogeneous across the microscope slides. We could not accurately measure the amount of calcified hyphae on the microscope slides, but we were able to document the presence or absence of calcification (**Table 3**). These results revealed that the water control slides did not have calcified hyphae regardless of vegetation type or parent material, with the exception of one slide in the grassland igneous and one slide in the forest limestone. However, the bare microscope glass slides, which also served as a control, did contained calcified hyphae in both desert sites and at both forest sites, but not for the majority of grassland samples. When growth medium was supplied, calcification occurred at both desert sites, both grassland sites (with the exception of one sample), and both forest sites. This growth-

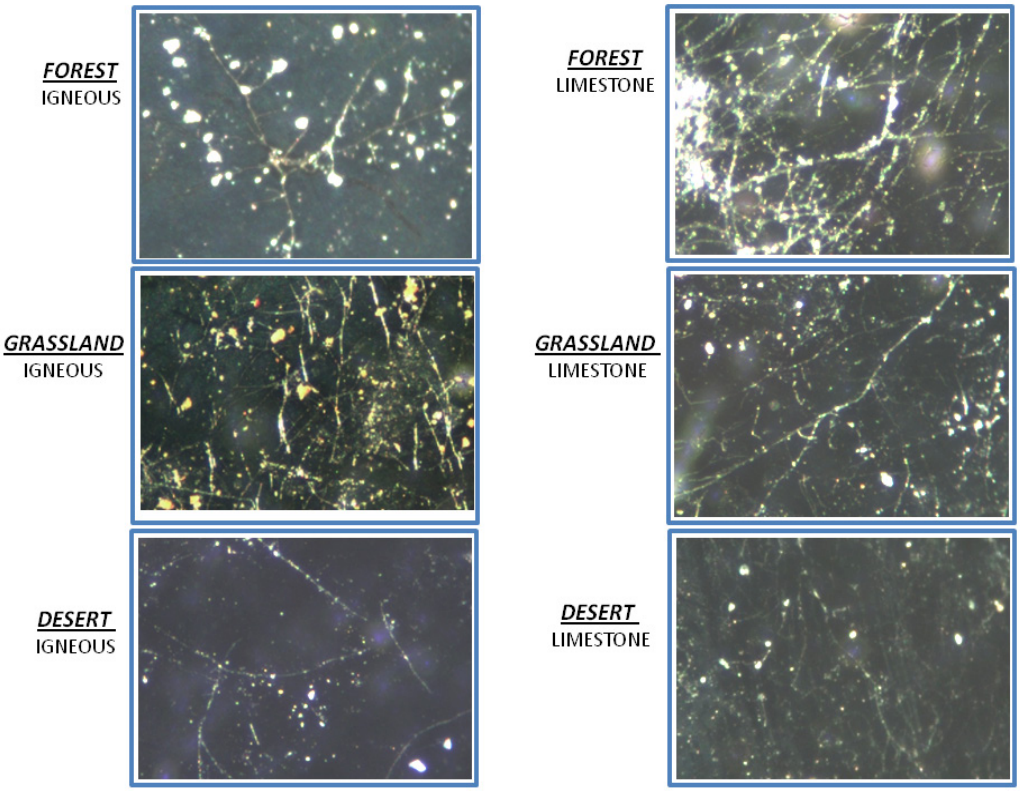

Figure 7. Photomicrographs of hyphae at each study site on cumulative slides January to February. Calcification occurs as linear white blocky zones along hyphae. (Photographs taken at 100x and crossed-polarized light).

Table 3. Presence (+) or absence (-) of calcified hyphae on microscope slides attached to the nylon wicks of the apparatuses used to deliver liquid B4 medium to soil

Month	Desert Igneous				Desert Limestone				Grassland Igneous				Grassland Limestone				Forest Igneous				Forest Limestone			
	Water[a]	Incr[b]	Cum[c]	Glass[d]	Water	Incr	Cum	Glass	Water	Incr	Cum	Glass	Water	Incr	Cum	Glass	Water	Incr	Cum	Glass	Water	Incr	Cum	Glass
JAN			+				+				+				+				+				+	
FEB			+			+	+			+	+			+	+			+	+			+	+	
MAR	-		+	+	-	+	+	+	-	+	+	+	-	+	+	-	-	+	+	+	-	+	+	+
APR	-	+	+	+	-	-	+	+	-	+	+	nf[e]	-	-	+	+	-	+	+	nf	-	+	+	+
MAY	-	+	+	+	-	+	+	+	-	+	+	-	nf	+	+	-	-	nf	+	+	nf	+	+	+
JUN	-	+	+	+	-	-	+	+	+	+	+	-	nf	+	+	-	-	+	+	+	+	+	+	+
JUL		+				+				+				+				+				+		

[a] "Water" apparatuses were filled with water instead of B4 medium to serve as a control.
[b] "Incr" apparatuses residing in soil for monthly increments of supplied with B4 medium.
[c] "Cum" apparatuses supplied with B4 medium placed in soil in December 2010 and harvested each month consecutively until July.
[d] "Glass" bare microscope slides that were buried and harvested at monthly intervals which also served as a control.
[e] Slides labeled "nf" were not found.

induced calcification occurred regardless of whether the slides were harvested at monthly increments or harvested at cumulative increments.

3.3. Carbonate formed in fiberglass cloths

Carbonate formed in fiberglass cloth samples during monthly and cumulative increments are shown in **Table 4** and **Figure 8**. Samples collected at monthly increments had an increase in April for the grassland limestone site and in May for the grassland igneous site. Carbonate formed in fiberglass at cumulative increments had a greater amount in the first month for three sites: desert igneous, forest limestone, and grassland limestone (**Figure 8**). Another peak can be seen at February for the grassland igneous site.

Total carbonate, which was obtained by summing the monthly values, is shown for February through June in **Figure 9**—the period when all sites have comparable measurements. The monthly-increment samples produced more carbonate than the cumulative-increment samples. Monthly-increment samples show more carbonate at the limestone sites than igneous site, but this trend was not found using the cumulative samples. Monthly-increment

Table 4. Amount of carbonate (expressed as inorganic carbon) generated in fiberglass cloth samples at each site collected at monthly and cumulative intervals

Month	Desert Igneous		Desert Limestone		Grassland Igneous		Grassland Limestone		Forest Igneous		Forest Limestone	
	Increm	Cumulative	Increm	Cumulative	Increm	Cumulative	Increm	Cumulative	Increm	Cumulative	Increm	Cumulative
	% IC[a]	% IC	% IC	% IC	% IC	% IC	% IC	% IC	% IC	% IC	% IC	% IC
JAN		0.85				0.20		0.63		0.00		1.56
FEB	0.44	0.00	0.31	0.00	0.00	0.66	0.00	0.00	0.41	0.00	0.00	0.00
MAR	0.04	0.23	0.11	0.13	0.19	0.07	0.61	0.00	0.25	0.00	0.57	0.00
APR	0.00	0.21	0.23	0.24	0.35	0.00	1.12	0.00	0.34	0.05	0.28	0.13
MAY	0.25	0.19	0.22	0.00	0.91	0.00	0.00	0.07	nf	0.00	0.27	0.00
JUN	0.44	0.41	0.48	0.46	0.01	0.06	0.40	0.11	0.22	0.00	0.62	0.00

[a] Weight percent inorganic carbon.

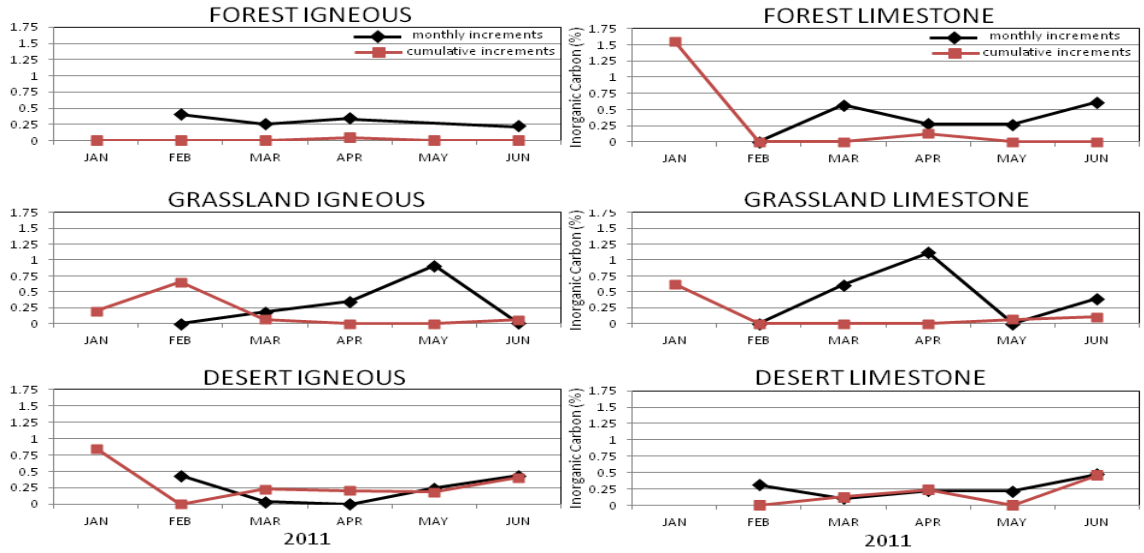

Figure 8. Monthly amounts of carbonate formed in fiberglass cloths during the experiment.

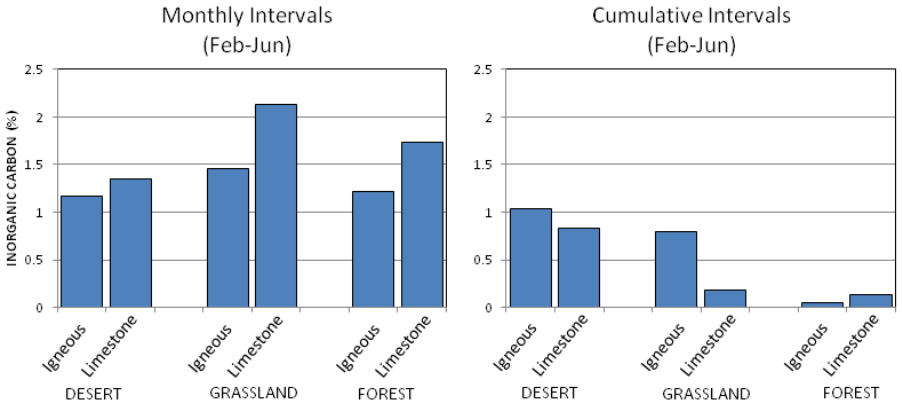

Figure 9. Total carbonate generated in the fiberglass cloths for the duration of the experiment (February to June 2011).

samples had more carbonate at the grassland sites, but the cumulative-increment samples had more carbonate at the desert site (Figure 9).

The reason that samples harvested at cumulative intervals have less total carbonate than those harvested at monthly intervals is unclear. Perhaps being in the soil for longer time periods caused carbonate that had formed earlier to dissolve later. If so, this result would suggest that carbonate biomineralization is a dynamic process that involves dissolution as well as precipitation. Short-term dissolution of carbonate has been documented elsewhere. Work by Smeck et al. (1968), for example, observed evidence for dynamic carbonate precipitation/dissolution in the Bk horizons of till-derived soils in western Ohio. They attributed the precipitation/dissolution cycles to seasonal water and leaching, which were followed by water deficit periods when carbonate precipitated.

3.4. Isotopic values

With respect to carbon isotopes, as mentioned earlier, there was not enough carbonate generated in the fiberglass cloths to determine $\delta^{13}C$ values. However, several natural values were determined on the vegetation, soil organic, and inorganic carbon from the study sites (**Table 2**). The desert igneous Btk horizon had a $\delta^{13}C$ value of inorganic carbon of -12‰ suggesting 0% C_4 vegetation based on equation (2). The same horizon had a $\delta^{13}C$ value of soil organic matter of -21‰. This suggests 34% C_4 vegetation based on equation (1). The desert igneous Bkkm organic value was -16‰, indicating 82% C_4; the inorganic value was -3‰, suggesting 64% C_4. The desert limestone Bkk1 had an organic $\delta^{13}C$ value -16‰ (82% C_4); the inorganic value was -7‰ (36% C_4). The grassland igneous site A horizon had $\delta^{13}C$ values of -15‰ for the organic (91% C_4), -5‰ for the inorganic (50% C_4), and -14‰ for the organic Btk (100% C_4), and -1‰ for the inorganic Btk (79% C_4). The forest igneous A horizon had a value of -22‰ (27% C_4). The forest limestone site A horizon had $\delta^{13}C$ values of -25‰ for the organic (0% C_4), -13‰ for the inorganic (0% C_4), and -23‰ for the organic Crk (18% C_4) and -9‰ for the inorganic Crk (21% C_4). These data show that in the grassland and forest sites the $\delta^{13}C$ values of both organic and inorganic carbon correspond well with the present dominant vegetation isotopic signatures. In the desert the $\delta^{13}C$ in surface horizons carries the signature of the dominant present C_3 vegetation, in contrast to the $\delta^{13}C$ in Bkkm horizons that suggest the dominance of the C_4 vegetation. This could be explained by the fact that the area was formerly dominated by C_4 grassland and later desertification caused the invasion of the area by C_3 desert shrubs (Monger et al. 2009). Therefore $\delta^{13}C$ signatures in the petrocalcic horizon are paleorelict features useful for explaining previous ecological conditions.

4. Conclusions

Traditionally, soil micromorphology studies of soil carbonate have been observational and based on thin section petrography, similar to mineralogy studies conducted by geologists (e.g., Drees and Wilding 1987; Khormali et al. 2006). Such observational studies have considered soil carbonate mainly in terms of inorganic chemistry. With the advent of SEM, progressively more attention has been given to a biogenic origin of soil carbonate, at least for a portion of soil carbonate. While this study builds on observational micromorphology, it could itself be categorized as *experimental micropedology*, in a manner similar to *experimental pedology*. As described by Hallsworth and Crawford (1965), "…it is now possible in pedology to put up a hypothesis and to submit it to the same kind of experimental investigation that has been responsible for the dramatic advances in other branches of science over the last fifty years." Based on an experimental micropedology approach, in addition to field observations, evidence from this study supports the following conclusions:

1. Microbial biomineralization is a widespread process in the deserts and grasslands of this study area and can extend into forests.

2. Calcification can be accelerated by adding liquid growth medium to soil *in situ*. But based on a substantial number of bare microscope slides inserted into soils, calcification by fungal hyphae is also a naturally occurring phenomenon that happens during a short time frame.

3. Indigenous microbial populations capable of biomineralizing carbonate in igneous soils when supplied with B4 liquid medium are not substantially different from neighboring population in limestone soils.

4. Amounts of carbonate formed in different bioclimatic zones are inconclusive. The samples harvested at monthly increments indicate grassland microbes formed more carbonate. However, samples harvested at cumulative increments indicate desert microbes made more carbonate. We can conclude, however, that samples harvested at monthly intervals

produced more carbonate than samples harvested at cumulative intervals.

5. With regard to carbon isotopes, the amounts of biogenic carbonate formed in the fiberglass traps were too low to measure $\delta^{13}C$ values. Therefore, no conclusions can be made about microbial fractionation of ^{13}C during biogenic precipitation. However, the natural values of the carbonates, which include the calcified microbes, may suggest a general conclusion. The A-horizons are more reflective of current vegetation than the subsoil horizons. Thus, these data support the notion that subsoil horizons have a "longer memory" than A horizons (Gerasimova and Lebedeva 2008).

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