Carbon Sequestration in Response to Grassland–Shrubland–Turfgrass Conversions and a Test for Carbonate Biomineralization in Desert Soils, New Mexico, USA

This study uses an experimental pedology approach to examine (i) how the conversion of native C_4 grassland to C_3 woody shrubs then to irrigated C_4 turfgrass affects both soil organic C (SOC) and soil inorganic C (SIC) and (ii) whether SIC can be enhanced by microbial biomineralization. Three sites were studied in the Chihuahuan Desert of New Mexico. At each site, SOC, SIC, and their δ^{13}C values were measured on control soil samples and compared to samples treated with liquid growth medium and Ca(OH)_2 at 10 and 40 cm. The treated samples were left to react for 1 mo in February, May, and August of 2014. Using a space-for-time substitution, soil organic C decreased when native grasslands converted to desert shrubs, then sharply increased after desert shrubs were converted to irrigated turfgrass. Most surprising, however, was the increase of SIC in the turfgrass site, which doubled in 6 yr. The δ^{13}C values of both SOC and SIC reflected the change from C_4 to C_3 then back to C_4 vegetation and showed how rapidly SOC and SIC can change their isotopic signatures. Soil inorganic C formation was slightly higher for the liquid growth medium, but no statistically significant differences were observed between the treatments and control samples. In addition, no biomineralization was observed with microscopy, perhaps because the 1-mo reaction time was too short and the amount applied was too small. Although SIC is typically viewed as a soil mineral that requires centuries to accumulate, our study indicates that SIC can be generated in months to years if the soil environment is suitable.

Abbreviations: SIC, soil inorganic C; SOC, soil organic C.

Sequestering C as SOC has achieved widespread recognition at high-profile climate conferences (Koch et al., 2015). Sequestering C as SIC, however, has achieved much less attention despite the fact that SIC is the second largest terrestrial C pool, containing at least 940 Pg C (Eswaran et al., 2000) compared to SOC’s >1500 Pg C (Post et al., 1982; Schlesinger and Bernhardt, 2013). Soil inorganic C is ubiquitous in dryland soils where it occurs at roughly 10 times the amount of SOC (Schlesinger, 1982).

In addition to SIC’s natural occurrence in desert soils, recent studies have measured increases in SIC as a result of agronomic management practices. In arid croplands in northwestern China, for example, it was documented that more C was sequestrated as SIC than SOC (Wu et al., 2009; Wang et al., 2014a, 2014b; Bughio et al., 2016). In Russia, chernozems used to grow crops contained more than twice the amount of SIC than chernozems still in native grass (Mikhailova and Post, 2006). In the US, SIC in the Imperial Valley increased after 85 yr of irrigated agriculture (Wu et al., 2008). Historically, however, SIC has been less thoroughly studied than SOC, probably because of the view that SIC (also known as CaCO_3 or calcite) is a soil mineral that remains unchanged by agronomic, rangeland, and forestry management techniques (Lal, 2004, 2008; Monger, 2014).

Core Ideas

- Soil carbonate is typically viewed as a soil mineral that requires centuries to accumulate; our study indicates that it can be generated in months to years.
- If the source of Ca is directly from silicate minerals, soil carbonate could be managed to sequester atmospheric CO_2.
- Carbon-13 values can change in less than a decade for both soil organic and inorganic C.
- This study uses an experimental pedology approach using controls, treatments, and replications.
Not only has SIC formation been suggested to occur more rapidly than traditionally thought, SIC is more biological than traditionally thought. Evidence to support a biological origin of SIC has come from (i) micromorphology studies of field specimens (Drees and Wilding, 1987; Klappa, 1979; Phillips et al., 1987 Verrecchia and Verrecchia, 1994), (ii) lab studies of soil microbes grown on agar (Boquet et al., 1973), (iii) soil column studies comparing soils inoculated with microorganisms versus sterile soils (Monger et al., 1991), and (iv) experimental micropedology studies using apparatus containing a liquid growth medium inserted in the soil (Khormali et al., 2014). This biological aspects of carbonate formation by microorganisms have been increasingly used for practical applications, such as the production of bio cement to protect ornamental stone (Rodriguez-Navarro et al., 2003), to strengthen construction material (De Maynck et al., 2010), and to sequester atmospheric CO2 (DeJong et al., 2010).

For C sequestration purposes, knowing whether SIC was formed in situ rather than being a detrital component of the parent material is important (Eshel et al., 2007). However, it is more essential to know the source of Ca in carbonates (Schlesinger, 1975). Calcium derived directly from silicate minerals (termed silicatic pedogenic carbonate) is a prerequisite for C sequestration, in contrast to Ca derived from preexisting carbonate minerals (termed calcitic pedogenic carbonate) (Monger et al., 2015).

This study uses experimental pedology to examine SOC and SIC sequestration in desert soils as a function of vegetation change and microbial biomineralization. Pedology per se is the study of soils as a natural phenomenon based on observational evidence and characterization data. Experimental pedology adds manipulative experiments with treatments, controls, and replications to test pedological hypotheses (e.g., Hallsworth and Crawford, 1965).

Specifically, our study addresses three questions: (i) Do SOC, SIC, and their δ13C values change when native C4 grass is replaced with C3 desert shrubs, which then are converted to irrigated C4 turfgrass? (ii) Can SIC be generated by microbial biomineralization when liquid growth medium is added to the soil in situ? (iii) If so, will the amount of SIC produced by biomineralization be greater than the amount of SIC produced by chemical precipitation from additions of Ca(OH)2?

MATERIALS AND METHODS

Study Site Description

The study was conducted in the southwestern US in southern New Mexico (Fig. 1). Site 1 (the native grass site) was located at the Chihuahuan Desert Rangeland Research Center Site 2 (the desert shrub site) was located in an undeveloped area with native vegetation on the New Mexico State University campus, and Site 3 (the turfgrass site) was located roughly 100 m east of the desert shrub site. The annual rainfall in the study region is 200 to 250 mm per year, with more than 50% falling in the summer monsoon season. The average air temperature is recorded as 17°C, giving rise to soils with aridic moisture and thermic temperature regimes (Gile et al., 1981).

At the native grass site, the dominant plant species is black grama [Bouteloua eriopoda (Torr.) Torr.], which uses the C4 photosynthetic pathway and has a C isotopic value of -14.0‰ ± 0.6 (Gallegos, 1999). This isolated grassland, which is only a few km2 in size, is a vestige of more expansive grasslands that existed a century and a half go in the region (Buffington and Herbel, 1965; Gibbens et al., 2005). This site also contains sparse amounts of C3 plants, such as soaptree yucca [Yucca elata (Engelm.) Engelm.] and Mormon tea (Ephedra torreyana S. Watson) separated from each other by tens of meters. In addition, C3 succulents appear at various times when weather conditions are favorable. Thus, some C3 C is put into the soil at this site but the vast amount comes from C4.

At the desert shrub site, the dominant plant species are creosotebush [Larrea tridentate (Sessé & Moc. ex DC.) Coville] and mesquite [Prosopis glandulosa Torr.] separated by bare ground with sparse cacti (Opuntia spp.) and small clumps of grasses (Aristida spp. and Bouteloua spp.), which appear after prolonged rains in the summer monsoon season. Both creosotebush and mesquite use the C4 photosynthetic pathway and have isotopic values of -24.2‰ ± 0.7 and -25.3‰ ± 0.4, respectively (Gallegos, 1999). However, according to land survey notes, the study region was dominated by native grasslands until the late 1800s, when woody shrubs replaced most of the grasses as a consequence of overgrazing and climate change (Buffington and Herbel, 1965; Gile, 1966; Peters et al., 2012). Thus we make the inference that the desert shrub site was dominated by C4 grasses 150 yr ago.

At the turfgrass site, a dense stand of bermudagrass (Cynodon dactylon L.) was established in 2008 (Serena et al., 2014). Bermudagrass, like black grama, uses the C4 photosynthetic pathway. The plot was irrigated daily with a pop-up sprinkler system (Hunter Rotator MP2000-90, Hunter Industries, San Marcos, CA) at 85% of reference evapotranspiration for short grass (Snyder and Eching, 2007), which amounted to 1200 mm during 2014. The irrigation water at that time had a pH of 7.6, an electrical conductivity of 0.6 dS m-1, and a Na adsorption ratio of 1.9 mmol1/2 L-1. Other chemical constituents included Ca (2.6 mmol L-1), Mg (0.8 mmol L-1), Na (2.1 mmol L-1), and bicarbonate (2.2 mmol L-1). During the research period, bermudagrass was mowed weekly to 2.0 cm with clippings removed and fertilizer applied to prevent nutrient stress. Fertilization was applied granularly in the form of urea and muriate of potash by means of a Gandy walk-behind drop spreader (Model 36H13; Gandy Corp., Owatonna, MN). Fertilization consisted of an annual total of 15 g N, 0 g P, and 4.2 g K m-2 applied every 6 wk at 3.75 g N m-2, 0 g P, and 1.04 g K m-2 from April to September.

Except for vegetation, the five soil-forming factors were held constant for the three sites as much as possible, especially soil age which has a major effect on the amount of SIC in the soil profile. The landform at the native grassland site is a fan-skirt and the geomorphic surface is the mid-Holocene age Organ surface (Gile et al., 1981). The landform at the desert shrub and adjacent turfgrass sites is a valley border terrace and the geomorphic
surface is the mid-Holocene Fillmore surface. Both geomorphic surfaces are characterized by having soils with weakly developed profiles. The physical properties of the soils are shown in Table 1. The parent material for both sites is alluvium dominated by igneous mineralogy, mainly quartz. Limestone detritus is not a component of the parent material at either site. Soil at the native grass site is classified as a coarse-loamy, mixed, superactive, thermic Ustic Haplocambid. Soil at both the C3 desert shrub and C4 turfgrass sites are classified as mixed, thermic Typic Torripsamments.

**Experimental Design, Treatments, and Sample Collection**

At each study site, soil samples were collected at 10 and 40 cm from three randomly located auger holes (15 cm in diameter) (Fig. 2). As soil was removed, it was placed in labeled buckets so it could be replaced at the depths from which it had been taken. Soil samples to be treated with the liquid growth medium and Ca(OH)2 were passed through a 2-mm sieve, poured into four porous plastic cylinders (3 by 5 cm), and then wrapped with nylon mesh fabric that was stretched to make holes large enough (>4 µm) to allow fungal hyphae to penetrate but small enough to keep soil from spilling out. Of the four samples collected at each depth, two were treated with B4 growth medium, one was treated with Ca(OH)2, and one was treated with deionized water to serve as a control.

**Table 1. Basic soil properties at study sites.**

<table>
<thead>
<tr>
<th>Site</th>
<th>Depth</th>
<th>Horizon</th>
<th>Sand</th>
<th>Silt</th>
<th>Clay</th>
<th>SOC$</th>
<th>SIC$</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native grass soil†</td>
<td>0–17</td>
<td>A</td>
<td>14.1</td>
<td>6.9</td>
<td>0.26</td>
<td>0.12</td>
<td>8.6</td>
<td>7.1</td>
</tr>
<tr>
<td>17–45</td>
<td>Bw1</td>
<td>83.2</td>
<td>11.3</td>
<td>5.5</td>
<td>0.12</td>
<td>0.14</td>
<td>trace</td>
<td>7.7</td>
</tr>
<tr>
<td>45–68</td>
<td>Bw2</td>
<td>79.9</td>
<td>13.8</td>
<td>6.3</td>
<td>0.14</td>
<td>2.7</td>
<td>8.2</td>
<td></td>
</tr>
<tr>
<td>58–122</td>
<td>Bk2</td>
<td>79.1</td>
<td>14</td>
<td>6.9</td>
<td>0.03</td>
<td>1</td>
<td>8.6</td>
<td></td>
</tr>
<tr>
<td>Desert shrub and turfgrass soil‡</td>
<td>0–13</td>
<td>A</td>
<td>90.1</td>
<td>5.1</td>
<td>0.14</td>
<td>0.1</td>
<td>8.6</td>
<td></td>
</tr>
<tr>
<td>13–43</td>
<td>B</td>
<td>90.1</td>
<td>4.8</td>
<td>5.1</td>
<td>0.11</td>
<td>0.5</td>
<td>8.6</td>
<td></td>
</tr>
<tr>
<td>43–64</td>
<td>C1ca</td>
<td>91.7</td>
<td>3.4</td>
<td>4.9</td>
<td>0.11</td>
<td>2.7</td>
<td>8.4</td>
<td></td>
</tr>
<tr>
<td>64–114</td>
<td>C2ca</td>
<td>93.7</td>
<td>2.9</td>
<td>3.4</td>
<td>0.05</td>
<td>2.4</td>
<td>8.4</td>
<td></td>
</tr>
</tbody>
</table>

†Data for the native grass soil are courtesy of the USDA-Natural Resources Conservation Services (S04NMO13–006).
‡Data for the desert shrub and turfgrass sites are from Gile et al. 1981.
§SOC, soil organic C; SIC, soil inorganic C.
The liquid B4 growth medium consisted of 15 g calcium acetate, 4.0 g yeast extract, and 10.0 g glucose per 1000 mL of distilled water, adjusted to pH 8.0 with NaOH (Boquet et al., 1973). The B4 liquid was applied aseptically by pipetting 5 mL of the sterile liquid onto the soil in the plastic container beneath the nylon mesh fabric. One of the two B4-treated samples was used for measuring SOC, SIC, and C isotopic values. The second was used for microscopy to determine if calcified microorganisms were present. For the microscopy examination, binocular microscopes was used and 1 M hydrochloric acid was added into tin capsules, and combusted in a C–N analyzer (EuroVector, Redavalle, Italy) connected to a continuous-flow isotope ratio mass spectrometer (IsoPrime-EA, Micromass, Stockport, UK). The δ13C values, which record the photosynthetic pathways of major plant groups and the effects of pCO2 (Schubert and Jahren, 2012), were expressed relative to the carbon isotopic composition of Vienna-PeeDee Belemnite. The standards used were the carbonate NBS-19 +1.95‰ and graphite USGS24 -16.049‰.

Other subsamples were not treated with acid, which provided a measure of total C (TC) and its δ13C values. The SOC was corrected by the weight loss from the acid treatment. Soil inorganic C was obtained from the difference between TC and SOC. The SIC δ13C values were determined via the following equation:

$$\delta^{13}C_{SIC} = \frac{TC \times \delta^{13}C_{TC} - SOC \times \delta^{13}C_{SOC}}{TC - SOC}$$  \[1\]

The validity of this method was tested and confirmed using known amounts of carbonate and graphite standards mixed in various proportions. The fraction of C4 carbon in SOC (F_{C4}) was estimated by the following equation (Boutton et al., 1999; Weems and Monger, 2012):

$$F_{C4} = \frac{\delta_{sample} - \delta_{C3}}{\delta_{C4} - \delta_{C3}},$$  \[2\]

where $\delta_{sample}$ is the δ13C of the bulk soil sample, $\delta_{C3}$ is the average δ13C value of C3 plants, $\delta_{C4}$ is the average δ13C value of the
C₄ plants. Values of -25‰ were used for C₃ plants and -14‰ for C₄ plants. The C₄ fraction based on pedogenic carbonate was estimated via Eq. [3]:

\[
F_{\text{C₄}} = \frac{\delta_{\text{sample ped carb}} - \delta_{\text{C₄ ped carb}}}{\delta_{\text{C₄ ped carb}} - \delta_{\text{C₃ ped carb}}},
\]

where \( \delta_{\text{sample ped carb}} \) is the \( \delta^{13}C \) of the carbonate in the soil sample, \( \delta_{\text{C₄ ped carb}} \) is the average \( \delta^{13}C \) value derived from C₄ plants, and \( \delta_{\text{C₃ ped carb}} \) is the average \( \delta^{13}C \) value derived from C₃ plants. Carbonate \( \delta^{13}C \) values of -12‰ were used for C₃ plants and +2‰ for C₄ plants.

**Soil CO₂ and Temperature Measurements**

Soil CO₂ levels were determined by using gas wells using a Vaisala CARBOCAP Hand-Held Carbon Dioxide Meter GM70 (Vaisala Oyj, Helsinki, Finland), which has a probe accuracy of ±1.5%. Gas wells were composed of polyvinylchloride pipe (12.7 mm i.d.) inserted into the soil at 10 and 40 cm depths. The end of the inserted pipe was open and had several small (2 mm) drill holes spaced 6 mm apart in the side walls up to 3 cm from the bottom (Buyanovsky and Wagner, 1983). The holes were then covered with a porous nylon cloth to keep the inside of the pipe clean. The top of each well was capped and a hose-barb was screwed into the cap. A 4-cm piece of neoprene tubing (3 mm i.d.) was attached to the barb and crimped with a clip to keep the well airtight. When a reading was taken, a Nafion membrane tube (Perma Pure, Lakewood, NJ) was attached to the crimped tube, the clip was removed, and soil air was pumped into the chamber for a CO₂ reading. Three readings were taken and averaged for the desert shrub and turfgrass sites and two readings for the native grass site. To determine diurnal CO₂ fluctuations, measurements were made at 3-hr intervals for 24 h on 26 May and 15 August.

Soil temperature was measured using NexSens Micro-T temperature sensors (iButton Thermochron DS1921G, Maxim Integrated, San Jose, CA). Readings were made at 1-hr increments and averaged for month-long periods for the durations when the sensors were in the ground. The sensors were placed at 10 and 40 cm depths.

**Calculations and Statistical Analysis**

Soil inorganic C accumulation rates (\( R_{\text{SIC}} \), in mg C m⁻² d⁻¹) were measured by the following equation:

\[
R_{\text{SIC}} = \frac{(\text{SIC} \times E_i - \text{SIC}_{\text{CK}} \times E_{\text{CK}}) \times H \times 10,000}{D}
\]

where SIC is the SIC content of the B₄ or Ca(OH)₂ treatments (g kg⁻¹), SICCK is the SIC content of the control, \( E_i \) is the soil bulk density (g cm⁻³), \( H \) is the length (1 cm), \( D \) is the duration of the experiment (days), and 10,000 is unit conversion factor.

ANOVARs were performed to evaluate the effect of soil temperature, soil CO₂, and precipitation on SIC accumulation. Following the ANOVAs, Fisher’s protected LSD was used to compare SIC and isotopic values between treatments. Student’s \( t \)-test was applied to evaluate the differences in the SIC accumulation rate between the B₄ and Ca(OH)₂ treatments. SPSS version 16.0 software (SPSS Inc., Chicago, IL) was used for the statistical analyses and SigmaPlot version 12.5 (Systat Software Inc., San Jose, CA) was used to create the graphs.

**RESULTS**

**Carbon Baseline**

**Soil Organic C**

Soil organic C at 10 cm at the native grass site was measured in February, May, and August and had an average value of 3.50 g kg⁻¹ with a SD of 0.98 (Table 2). The desert shrub site had an average value of 1.52 g kg⁻¹ with a SD of 0.29 and the turfgrass site had an average value of 2.82 g kg⁻¹ with a SD of 1.25. The SOC values at 40 cm were lower: 2.30, 1.46, 1.78 g kg⁻¹, respectively (Table 2).

On the basis of the inference that the native grass site represents SOC that would have existed at the desert shrub site in the mid-1800s (Buffington and Herbel, 1965; Gile, 1966; Gibbens et al., 2005), then a drop in organic C occurred when shrubs replaced the grassland, especially at the 10 cm depth (Fig. 3A,B). In contrast, converting the desert shrub site to turfgrass increased the amount of C in 6 yr, especially at the 10 cm depth. Isotopically, SOC \( \delta^{13}C \) values confirm the change from C₄ grass to C₃ shrubs then back to C₄ grass. The \( \delta^{13}C \) values of SOC at the native grass site at 10 cm depth dropped from an average of -16.5% in the native grassland to -22.5% in the desert shrub site, which then rose to -17.3% in the turfgrass site (Fig. 3C). Using Eq. [2], this equates to a drop from 77% organic C from C₄ vegetation in the native grass to 22% C₄ at the desert shrub site, followed by a rise to 70% in the turfgrass site (Fig. 3C). A similar trend occurred at the 40 cm depth (Fig. 3D). The \( \delta^{13}C \) values at the turfgrass site demonstrate how rapidly SOC can convert from C₃ to C₄ signatures.

**Soil Inorganic C**

Soil inorganic C at 10 cm in the native grass site had an average value of 1.22 g kg⁻¹ with a SD of 0.04 (Table 2). The desert shrub site had an average value of 1.92 g kg⁻¹ with a SD of 1.69. The turfgrass site had an average value of 4.41 g kg⁻¹ with a SD of 1.60. In contrast to the SOC values, those of SIC were higher at 10 cm rather than lower (Table 2), which is typical of desert soils in the study region (Gile et al., 1981).

On the basis of the inference that the native grass site represents the amount of SIC that existed at the desert shrub site in the mid-1800s, an increase in SIC occurred when shrubs replaced the grassland (Fig. 4A,B). With regard to the conversion of desert shrubs to turfgrass, a notable increase in SIC at 10 cm rather than lower (Table 2), which is typical of desert soils in the study region (Gile et al., 1981).

Isotopically, SIC \( \delta^{13}C \) values also reflect the change from C₃ shrubs and C₄ grass. The SIC content at the native grass site were too low to obtain accurate \( \delta^{13}C \) values, given the limitations of our continuous flow isotope ratio mass spectrometer. However,
at the desert shrub site, a value of $-12.7$ rose to $-5.9\%$ in the adjacent turfgrass site (Fig. 4C). On the basis of Eq. [3], this equates to an absence of C$_4$ at the desert shrub site, followed by a rise to 43% in the turfgrass site in 6 yr (Fig. 4C). Little change in the isotope values occurred at the 40 cm depth (Fig. 4D). As for SOC, the 6$^{13}$C values of SIC suggest a rapid change in isotopic signatures when C$_4$ vegetation replaces C$_3$ vegetation.

**Treatment Effects**

**Soil Organic C**

Did SOC change when the B$_4$ and Ca(OH)$_2$ treatments were added to soil? Regardless of season or depth, the amount of SOC at the native grass, desert shrub, or turfgrass sites were little affected by either the B$_4$ growth medium or Ca(OH)$_2$ (Fig. 5). This was expected, since SOC is primarily dependent on C inputs from plant growth and neither treatment was large enough or had enough time to affect plants.

Isotopically, SOC was also little affected by either the B$_4$ or Ca(OH)$_2$ treatment (Fig. 5). More variability occurred among sites than among treatments or seasons. For example, at 10 cm, the control samples at the desert shrub site averaged across February, May, and August were close to the pure C$_3$ line of -25‰ (Fig. 5C). In contrast, the native grass and turfgrass sites at 10 cm depth were about midway between pure C$_3$ and C$_4$ (Fig. 5A,E). Similarly, at 40 cm depth, the desert shrub site had a 6$^{13}$C value of -20.2‰, which was closer to the pure C$_3$ value (Fig. 5D) than the native grass or turfgrass sites, which were closer to pure C$_4$ (Fig. 5B,F).

**Table 2. Natural abundance of soil organic C (SOC) and soil inorganic C (SIC) in bulk samples and their isotopic compositions.**

<table>
<thead>
<tr>
<th>Month</th>
<th>SOC (g kg$^{-1}$)</th>
<th>6$^{13}$C$_{SOC}$ (‰)</th>
<th>SIC (g kg$^{-1}$)</th>
<th>6$^{13}$C$_{SIC}$ (‰)</th>
<th>Soil temp.† (°C)</th>
<th>Soil CO$_2$‡ (μL L$^{-1}$)</th>
<th>Ppt.§ (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 cm</td>
<td>40 cm</td>
<td>10 cm</td>
<td>40 cm</td>
<td>10 cm</td>
<td>40 cm</td>
<td>10 cm</td>
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<tr>
<td>Native grass</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feb.</td>
<td>4.76</td>
<td>2.93</td>
<td>-17.5</td>
<td>-16.3</td>
<td>-</td>
<td>-</td>
<td>17.3</td>
</tr>
<tr>
<td>May</td>
<td>3.80</td>
<td>2.00</td>
<td>-15.9</td>
<td>-14.2</td>
<td>-</td>
<td>0.50</td>
<td>35.6</td>
</tr>
<tr>
<td>Aug.</td>
<td>1.99</td>
<td>1.45</td>
<td>-15.8</td>
<td>-14.5</td>
<td>-</td>
<td>0.98</td>
<td>30.5</td>
</tr>
<tr>
<td>Avg.</td>
<td>3.50</td>
<td>2.30</td>
<td>-16.54</td>
<td>-15.45</td>
<td>-</td>
<td>-</td>
<td>17.3</td>
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<tr>
<td>Desert shrub</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Feb.</td>
<td>1.79</td>
<td>1.37</td>
<td>-23.4</td>
<td>-21.2</td>
<td>0.79</td>
<td>3.75</td>
<td>14.5</td>
</tr>
<tr>
<td>May</td>
<td>1.38</td>
<td>1.01</td>
<td>-23.1</td>
<td>-20.2</td>
<td>1.52</td>
<td>4.56</td>
<td>31.3</td>
</tr>
<tr>
<td>Aug.</td>
<td>1.93</td>
<td>1.54</td>
<td>-23.0</td>
<td>-19.0</td>
<td>1.40</td>
<td>8.07</td>
<td>31.0</td>
</tr>
<tr>
<td>Avg.</td>
<td>1.52</td>
<td>1.46</td>
<td>-22.54</td>
<td>-20.18</td>
<td>-</td>
<td>-</td>
<td>17.3</td>
</tr>
<tr>
<td>Turfgrass</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Feb.</td>
<td>2.23</td>
<td>1.68</td>
<td>-19.4</td>
<td>-18.6</td>
<td>3.24</td>
<td>5.54</td>
<td>11.4</td>
</tr>
<tr>
<td>May</td>
<td>1.85</td>
<td>0.90</td>
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<td>-17.1</td>
<td>4.44</td>
<td>6.03</td>
<td>20.1</td>
</tr>
<tr>
<td>Aug.</td>
<td>2.15</td>
<td>1.66</td>
<td>-18.0</td>
<td>-18.2</td>
<td>3.60</td>
<td>6.51</td>
<td>25.5</td>
</tr>
<tr>
<td>Avg.</td>
<td>2.82</td>
<td>1.78</td>
<td>-17.27</td>
<td>-17.57</td>
<td>-</td>
<td>-</td>
<td>17.3</td>
</tr>
<tr>
<td>SD</td>
<td>0.29</td>
<td>0.33</td>
<td>1.28</td>
<td>0.99</td>
<td>1.69</td>
<td>2.90</td>
<td>1.88</td>
</tr>
</tbody>
</table>

† Average of readings taken at 1-hr increments for the month-long duration of the experiment.
‡ An average of three readings for desert shrub and turfgrass sites and two readings for native grass were taken on the day samples were harvested at the end of the month.
§ Total precipitation for the duration of each monthly experiment.
¶ nm, not measured, which indicates that this value was not measured because of instrument failure; –, not detected.
Fig. 3. Soil organic C (SOC) graphs plotting time versus SOC amounts at (A) 10 and (B) 40 cm. The dashed line indicates the inference that the
desert shrub site was formerly a native grassland in the mid-1800s (Buffington and Herbel, 1965; Gile, 1966). Soil organic C graphs plotting $\delta^{13}$C
values at (C) 10 and (D) 40 cm and percentage of C from $C_4$ vegetation.

Fig. 4. Soil inorganic C (SIC) graphs plotting time versus SIC amounts at (A) 10 and (B) 40 cm. The dashed line indicates the inference that the
desert shrub site was formerly a native grassland in the mid-1800s (Buffington and Herbel, 1965; Gile, 1966). Soil inorganic C graphs plotting $\delta^{13}$C
values at (C) 10 and (D) 40 cm and percentage of C from $C_4$ vegetation.
Did SIC change when the B4 and Ca(OH)$_2$ treatments were added to soil? The SIC response to the treatments was more variable than the SOC response but was still minor (Fig. 6). The treatment response was not apparent at either 10 or 40 cm (Fig. 6A,B). For the other two sites containing SIC, the largest response was at 10 cm depth in May at the desert shrub site (Fig. 6C). The Ca(OH)$_2$ treatment at 10 cm depth also had its

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**Soil Inorganic C**

**Fig. 5.** Soil organic C (SOC) amounts and $\delta^{13}$C values in soil samples at (A,C,E) 10 and (B,D,F) 40 cm for the three study sites. (A,B) Native grass; (C,D) desert shrub; (E,F) turfgrass. The blue line shows the average C and $\delta^{13}$C values in control samples across February, May, and August. Red dashed lines locate the position of pure $C_3$ and $C_4$ vegetation.

**Fig. 6.** Soil inorganic C (SIC) amounts and $\delta^{13}$C values in soil samples at (A,C,E) 10 (B,D,F) 40 cm for the three study sites. (A,B) Native grass; (C,D) desert shrub; (E,F) turfgrass. The blue line shows the average C and $\delta^{13}$C values in control samples across February, May, and August. Red dashed lines locate the position of pure $C_3$ and $C_4$ vegetation. mass spec, mass spectrometry.
Ca(OH)₂ treatments than their amount of SIC, especially at the May (Fig. 6C). The Ca(OH)₂ treatment, on the other hand, was higher than the control for February and August but lower for May (Fig. 6C). The Ca(OH)₂ treatment, on the other hand, was lower for February and May but higher in August. Still, all sites lacked statistically significant differences at the \( p < 0.05 \) level.

Microscopy

Microscopy was used as additional evidence to determine whether SIC formed after the B4 medium was added to the soil. As shown in Fig. 2, one of the two B4 treatments at both 10 and 40 cm was examined with light and electron microscopy for calcified microorganisms in the form of calcified roots, fungal hyphae, or bacteria. Fungal biomineralization, for example, would be expected to appear like the image in Fig. 7A, which is common in the desert soils of the study area (Monger et al., 1991). The results, however, were negative. The closest example of biomineralization that could be found after examining all samples is shown in Fig. 7B. However, even this specimen displayed only weak, if any, evidence of calcification.

Soil Inorganic C Accumulation Rates

In addition to our examination of the total amounts of SIC produced by the addition of B4 and Ca(OH)₂, we also re-examined the SIC data in the form of accumulation rates using Eq. [4], which magnifies the differences. In general, the accumulation under B4 was slightly greater than under Ca(OH)₂ but the variability was high and no statistically significant differences were found. Both B4 and Ca(OH)₂ typically had higher accumulation rates than the controls (Fig. 8A–F), except for a few cases when it was lower, which indicates dissolution in comparison to the control samples.

Correlations of SIC in Treated Samples to Physical Conditions

Soil inorganic C formation and dissolution is controlled by Ca²⁺ and CO₃²⁻ activity. These variables operate within the context of soil temperature, precipitation, and CO₂ concentrations (Breecker et al., 2009), which exert important controls over SIC formation in dryland soils in the study region. Soil inorganic C generated by B4 and Ca(OH)₂ treatments, therefore, was examined in light of these variables.

Soil temperatures at 10 cm during February, May, and August were similar between the native grass and desert shrub sites but, owing to the effect of irrigation, were 5 to 10°C cooler for the turfgrass site (Table 2; Fig. 8G–I). Temperatures at 40 cm followed a similar trend to those at 10 cm at all sites but were slightly cooler.

Precipitation, like temperature, was similar for the native grass and desert shrub sites but much greater for the turfgrass site because of the irrigation. The native grass site received 54 mm and the desert shrub site received 56 mm, in contrast to the turfgrass site, which received 1200 mm (Table 2; Fig. 8I, K, and G). In essence, irrigation changed the turfgrass site from a soil with an aridic moisture regime to a soil with an udic moisture regime.

Carbon dioxide concentrations closely followed soil moisture concentrations, as revealed by comparing Fig. 8G, 8H, and 8I with Fig. 8J, 8K, and 8L. At 10 cm depth, CO₂ ranged from 600 to 2050 \( \mu \text{L} \text{L}^{-1} \) at the native grass site, from 590 to 1670 \( \mu \text{L} \text{L}^{-1} \) at the desert shrub site, and from 2075 to 11,117 \( \mu \text{L} \text{L}^{-1} \) at the turfgrass site. At 40 cm depth, the increase was even more noticeable: CO₂ ranged from 690 to 3080 \( \mu \text{L} \text{L}^{-1} \) at the native grass site, from 590 to 1945 \( \mu \text{L} \text{L}^{-1} \) at the desert shrub site, and from 2900 to 20,051 \( \mu \text{L} \text{L}^{-1} \) at the turfgrass site.

To put seasonal CO₂ variability in the context of diurnal variability, CO₂ was measured during a 24-hr period at the desert shrub and turfgrass sites on two dates: first in May (the hot, dry season) and again in August (the hot, moist monsoon season). These measurements revealed that diurnal CO₂ variability was small compared to seasonal increases. At the desert shrub site in May, diurnal values ranged from approximately 350 \( \mu \text{L} \text{L}^{-1} \) at 10 cm depth to 650 \( \mu \text{L} \text{L}^{-1} \) at 40 cm depth, in contrast to the August values, which ranged from 1100 \( \mu \text{L} \text{L}^{-1} \) at 10 cm to 1800 \( \mu \text{L} \text{L}^{-1} \) at 40 cm (Fig. 9A). At the turfgrass site, CO₂ was much higher and the concentration at 10 cm remained below the concentration at 40 cm, unlike the desert shrub site. In May, diurnal values at the turfgrass site ranged from 7500 \( \mu \text{L} \text{L}^{-1} \) at 10 cm depth to 17,000 \( \mu \text{L} \text{L}^{-1} \) at 40 cm, in contrast to the August values, which ranged from 11,000 \( \mu \text{L} \text{L}^{-1} \) at 10 cm and 21,000 \( \mu \text{L} \text{L}^{-1} \) at 40 cm (Fig. 9B).

Although it is well known that temperature, rainfall, and soil CO₂ are important factors for carbonate formation, an attempt to find statistically significant correlations among these variables individually was unsuccessful (\( p < 0.05 \)). However, taken together, these factors were statistically significant (Table 3).
The statistical analysis was repeated using a stepwise procedure for each factor. Still, there was no single factor effect. Thus, SIC in this study was regulated not by single parameters but by multiple factors.

DISCUSSION

How valid is the conclusion that a decline in SOC accompanied the conversion of native grasslands to desert shrub, as indicated by Fig. 3A? First, we must emphasize that we substituted space for time by making the inference that the vestigial grassland at Site 1 represents how the desert shrub soil at Site 2 would have appeared 150 yr ago. Still, such a loss of SOC would be expected based on the typical amount of C in a grassland soil versus the typical amount in a desert shrub soil. The SOC in the remaining grasslands of the study region is about 1% compared to less than 0.5% in local shrublands soils (Gile et al., 1981). However, it must be pointed out that SOC in a grassland is more homogeneously distributed at the landscape scale than SOC in a shrubland. Soil directly beneath some shrubs can actually have more SOC than grassland soils (Throop and Archer, 2008).

It has also been pointed out that although losses of SOC have occurred with the grass–shrub conversion, C storage of the ecosystem as a whole has changed little, especially in light of the extensive rooting system of the woody shrubs (Schlesinger and Pilmanis, 1998).

More striking was the increase in SOC resulting from the desert site being converted to turfgrass. In this case, we are making the inference that the turfgrass soil had the same C as the neighboring desert shrub soil before 2008. Given (i) the close proximity of the adjacent sites (100 m), (ii) the detailed soil mapping in the study area, and (iii) the uniform nature of the Torripsamment parent material, this seems to be a reasonable inference. In fact, such increases in SOC have been documented for almost 50 yr in the study area (Ruhe, 1967). At the time of Ruhe’s study, some areas of New Mexico State University had been in turfgrass since the 1890s. Soil organic C in these irrigated lawns were found to be 3.7 to 12.5 times greater than in the neighboring desert shrub soils.

Carbon isotopes have been used to estimate turnover rates of organic matter for at least three decades (e.g., Balesdent et al., 1987; Boutton, 1991). In this study, the δ13C values of SOC at 10 cm depth indicate a turnover from 77% C4 in the native
Soil inorganic C, in contrast to SOC, is typically viewed as a long-term soil component that forms according to the reaction
\[ \text{Ca}^{2+} + 2\text{HCO}_3^- \rightarrow \text{CaCO}_3 + \text{CO}_2 + \text{H}_2\text{O}. \]

In the study area, there is much soil-geomorphic evidence to support the view that SIC is a long-term mineral (Hawley et al., 1976). Radiocarbon dates that go back as far as 30,630 yr before the present also support this view (Monger et al., 1998). However, there also appears to be a superimposed dynamic carbonate component in native soils. In managed soils, like at the turfgrass site, this study suggests the possibility that SIC can be purposefully managed.

Soil inorganic C $^{13}$C values are more commonly used to investigate paleoclimates than turnover rates (e.g., Cerling, 1984; Rabenhorst et al., 1984; Landi et al., 2003; Stevenson et al., 2005). Soil inorganic C values in this study, however, suggest that carbonate turnover rates might be surprisingly fast when $C_4$ grasses replace $C_3$ grass or vice versa. Hence, $^{13}$C values can be useful for investigating the neoformation of carbonate in managed systems (e.g., Li et al., 2007; Mi et al., 2008; Wang et al., 2014a).

The result that neither the B4 nor Ca(OH)$_2$ treatment had a significant effect on the amount of $^{13}$C values of SOC was not surprising because neither treatment had enough time to affect plant growth or microbial decomposition of the existing organic matter. The treatments did, however, have a slightly greater impact on SIC, as shown by their greater variability. Both treatments caused slightly greater amounts of SIC to form than in the controls but the differences were not statistically significant.

Within the slight increase stimulated by the treatments, B4 produced more SIC than Ca(OH)$_2$. The increased SIC resulting from the Ca(OH)$_2$ treatments is in agreement with many other researchers who have successfully modeled the precipitation and dissolution of Ca(OH)$_2$ by abiotic pathways (e.g., Hirmas et al., 2010). The increased SIC from the B4 treatments was expected to result from biomineralization via metabolic processes that mediate pH, CO$_2$, and Ca (Phillips et al., 1987; Frankel and Bazylinski, 2003). However, no microbial calcification was found via microscopy.

Measurements of soil temperature, moisture, and CO$_2$ in similar soils in New Mexico by Breecker et al. (2009) led these authors to conclude that pedogenic carbonate is formed in the warm, dry conditions during May when CO$_2$ concentrations are low. Consequently, the $^{13}$C values of pedogenic carbonates would not record the mean growing season vegetation, as typically assumed, but would instead record disproportionately more $C_4$ vegetation. Other studies have also shown a seasonal effect on the timing of carbonate formation, especially as a function of depth and when soil drying occurs (Ringham et al., 2016) and when respiration rates are higher (Oerter and Amundson, 2016). Our results do not support these conclusion, given the insignificant differences in SIC formation we observed among seasons. However, neither does our study dispute their conclusions because of the short duration of our experiment.

The observed average $^{13}$C values of pedogenic carbonates at 10 cm at the desert shrub site was -12.7‰, which is lower than the values below -11‰ commonly observed for carbonates in the study area (Deutz et al., 2002; Liu et al., 2007). Why are the values at this one location so low? Given a theoretical enrichment from diffusional and temperature-driven fractionation at 23°C (Cerling and Quade, 1993), we would expect values of around -10‰. However, previous studies of carbonate formed on the roots of creosotebush have shown values as low as -18.5‰ (Monger et al., 2009). The samples at 10 cm at Site 2 (the desert shrub site) were collected in soil dominated by fine creosotebush roots, which might explain the low values, although further investigation is needed. We do not, however, attribute the low values to analytical error because (i) the other SIC $^{13}$C values are reasonable and (ii) the analytic method used for obtaining these values was tested and verified with standards.

What role did Ca in irrigation water play in the observed increase in SIC at the turfgrass site? The concentration of Ca in the irrigation water was 2.6 mmol L$^{-1}$ and 1200 mm of irrigation was added in 2014. If we assume the same concentration and rate of irrigation for the previous 6 yr, then 749 g of Ca would have been added per m$^2$. If all of this Ca reacted to form CaCO$_3$ in the upper 10 cm, then 1872 g of CaCO$_3$ would have been added to the top 10 cm of each m$^2$ of soil. Given a bulk density of 1.4 g cm$^{-3}$, this amount could result in an increase of 1.3% CaCO$_3$. Of course, some Ca would have leached and some would have been taken up by plants. Still, on a theoretical basis, Ca in irrigation water could account for the observed SIC.

Can irrigated agriculture sequester atmospheric CO$_2$ in the broad context of upstream CO$_2$ emissions? Any pumping of irrigation water that involves fossil fuel would need to be considered. Any processing and transportation of Ca fertilizer that involved fossil fuel would also need to be considered. Any Ca from preexisting CaCO$_3$ would need to be considered because it negates sequestration. Given these limitations, if irrigation is gravity-fed and if Ca in the irrigation water comes from the

### Table 3. ANOVA for soil inorganic C accumulation rate as a function of temperature (T), soil CO$_2$ concentration (SCO$_2$), and precipitation (P).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Sum of squares</th>
<th>Mean of squares</th>
<th>F-value</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>T</td>
<td>1</td>
<td>65,896</td>
<td>65,896</td>
<td>3.78</td>
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<td>SCO$_2$</td>
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<tr>
<td>P</td>
<td>1</td>
<td>23,151</td>
<td>23,151</td>
<td>1.33</td>
<td>0.260</td>
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<td>T × SCO$_2$</td>
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<td>37,997</td>
<td>37,997</td>
<td>2.18</td>
<td>0.152</td>
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<tr>
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<td>6,326</td>
<td>6,326</td>
<td>0.36</td>
<td>0.552</td>
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<td>SCO$_2$ × P</td>
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<td>0.007</td>
</tr>
<tr>
<td>T × SCO$_2$ × P</td>
<td>1</td>
<td>102,334</td>
<td>102,334</td>
<td>5.87</td>
<td>0.023</td>
</tr>
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chemical weathering of silicates, then some of the CO₂ from root and microbial respiration could be sequestered as CaCO₃.

CONCLUSIONS

The main result of this study reveals that converting desert shrubs to turfgrass can increase SIC in less than 10 yr, which is unexpected, given the common view that SIC is a mineral that takes centuries to millennia to accumulate in soil. This experiment, therefore, supports other studies (e.g., Wang et al., 2014b), suggesting that SIC, like SOC, might be managed to sequester atmospheric CO₂ in desert environments. However, before these results can be generalized to other soils, it needs to be demonstrated that the source of Ca is derived directly from silicate minerals rather than preexisting carbonate, that the carbonate is durable and not subject to rapid dissolution, and that upstream CO₂ emissions are accounted for (Schlesinger, 1999).

The test for microbial biomineralization was negative. Although the liquid growth medium produced slightly more SIC, differences were neither statistically different between treatments nor between treatments and controls. In addition, no microbial calcification was found by using electron microscopy. Further studies are needed that use more liquid growth medium and allow it to react for longer periods than 1 mo.

The C isotope results of this experimental pedology study have implications for paleoclimatic studies by increasing our understanding of “soil memory” (Targulian and Goryachkin, 2004; Monger and Rachal, 2013). First, this study shows that δ¹³C values can change in less than a decade for both SOC and SIC. Thus soils, like those in this study, can be overprinted and described as having “short memories.” Second, comparing the relative changes in δ¹³C values between the 10-cm samples and the 40-cm samples supports the observation that subsoil B horizons have “longer memories” than surface A horizons (Gerasimova and Lebedeva, 2008). Thus an experimental pedology approach can supplement traditional paleopedology studies that must rely primarily on observation, deduction, and induction.

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