EFFECTS OF ELEVATED ATMOSPHERIC CO\textsubscript{2} ON SOIL ORGANIC CARBON STOCKS AND STABILITY IN THE MOJAVE DESERT

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EVALUATING ATMOSPHERIC CO2 ON SOIL ORGANIC CARBON STOCKS AND STABILITY IN THE MOJAVE DESERT

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Cornell University 2022

Elevated atmospheric CO2 (eCO2) is perturbing the global carbon (C) cycle. There is high uncertainty in how this disturbance will impact soil organic carbon (SOC) stocks. Quantifying SOC stocks and susceptibility to disturbance in deserts is critical because of the widespread geographic distribution of arid ecosystems. This thesis investigates the long-term effects of eCO2 on SOC stocks at the Nevada Desert Free-Air Carbon Dioxide Enrichment Facility (NDFF).

Chapter one compares two methods of soil inorganic carbon removal on SOC stock measurements and how these methods differ by CO2 treatment. We find that 30% of SOC can be lost using acid washing relative to fumigation and that this effect is greatest in soils from control plots at NDFF. These results indicate that SOC stabilization may change after long-term exposure to eCO2. Chapter two directly applies the finding from chapter one to understand variation in SOC stock measurements from NDFF across multiple studies. The result of a direct comparison demonstrates how statistical choices, acid pretreatment methods, and heterogeneity of soils can impact our understanding of eCO2 effects at NDFF.
Chapter three quantifies changes in SOC stability using a two-pool density fractionation. We find that eCO$_2$ decreases fast-cycling particulate organic matter and does not affect slower-cycling mineral associated organic matter. Chapter four quantifies eCO$_2$ effects on microbial mineralization in NDFF soils. We measure soil C priming with a $^{13}$C-labelled glutamic acid substrate using a short-term incubation and find eCO$_2$ effects are strongest in water amended soils. Priming is not affected by eCO$_2$ in water or C amended soils these conditions but is negatively correlated with SOC concentration.

Together, these chapters indicate NDFF soils are primarily C limited and the increase in organic C availability from eCO$_2$ treatment drives loss of SOC stocks. Addition of C in the form of acidic root exudates may destabilize organic matter and promote mineralization via increased microbial access, particularly from the C-rich particulate organic matter pool. These results emphasize the need to study multiple drivers of global change in arid ecosystem to fully understand impacts on SOC stocks and the global C cycle.
BIOGRAPHICAL SKETCH

Kelsey Jensen grew up in Williston, Vermont where she developed an interest in science through enthusiastic middle school and high school teachers. At Champlain Valley Union High School, she was captain of the field hockey team and debate team. Her team won a state championship in field hockey her junior year and as a senior she was named All-State Player of the Year. Kelsey was a National Merit Scholar and an honorable mention for the Presidential Award. She pursued her athletic and academic interests at Colgate University where she played four years of Varsity field hockey, captaining the team in her senior year, and double majored in chemistry and environmental studies. Kelsey was named to the Academic All Patriot League in 2012 and 2013 and was awarded the Colgate University Gottesman Award for Excellence in Athletics and Science at convocation.

Kelsey started scientific research in Dr. Anthony Chianese’s lab at Colgate following her sophomore year. She studied organometallic catalysis for three years, writing a senior honors thesis, presenting a poster at the American Chemical Society Conference, and co-authoring two papers published in the Journal of Organometallic Chemistry. In her senior year, Kelsey also joined a research team led by Dr. Catherine Cardelús conducting conservation research in Ethiopia’s sacred church forests. She wrote a thesis for her Environmental Studies major on the soil status of these forest and continued working on this project for several years, returning to Ethiopia as a field technician in 2016 and co-authoring a paper on soil health.

In 2016, Kelsey started her Doctoral research with Dr. Jed Sparks as an IGERT Fellow. In addition to service positions within the department, Kelsey served as President of Cornell Biogeochemistry Graduate Student Association for two years. Kelsey worked as a teaching assistant for five different classes, including two years
with Investigative Biology Labs, and mentored two undergraduate research assistants for several years. One student, Megan Eno, completed an honors thesis for Arts & Science Biology on her research. In addition to time in the lab, Kelsey participated in several Congressional outreach events to advocate for soil health policies at the national level through Cornell’s Advancing Science and Policy program and the Soil Science Society of American.

In her seven years living in Ithaca, Kelsey played in the town soccer league, coached the Cornell Club Field Hockey program, and started trail running with the Sea Pigs running team. Kelsey also continued to volunteer with the Vermont HOBY Leadership Program throughout her PhD studies, serving two years as the Leadership Seminar Chair, in addition to roles as Alumni Director and Vice President of the board.
For my parents, Jeanne and Dave
ACKNOWLEDGMENTS

This thesis would not exist without my advisor, Jed Sparks, and I am incredibly grateful to him for accepting me into his lab when I was an aspiring ecologist. Jed has been a committed advisor and mentor throughout my entire dissertation, knowing when and how to challenge and support me to maximize my growth as an independent scientist. I must also thank Kim Sparks who ensures that the Sparks Lab and Isotope Lab run smoothly and is always willing to help troubleshoot an experimental method. Kim is always there to talk about data or about your day and I would not have made it through a PhD consisting entirely of lab work without her company and advice.

I also managed to have a ton of fun in between meetings and experiments thanks to the community that Jed and Kim foster. I will look back fondly on my time in the Sparks lab, and all of the friends, jack-o-lanterns, and memories I made there. From the Sparks lab I would like to thank Fiona Soper, Ellie Goud, Ben Johnson, Amelia Weiss, Sudan Kariuki, Dan Petticord, and Cam Blevins for their mentorship, intellectual curiosity, feedback, patience, and friendship.

I would like to thank my committee members, Christy Goodale, Enid Martinez and Stuart Grandy (University of New Hampshire). Christy was always willing and excited to help me interpret my data in her lab meetings. Enid helped me with the basic of soil science and allowed me to discuss methods with her lab group for several years. Stuart invited me to visit his lab to learn new methods and continued to discuss my thesis with me for many years over Zoom. I learned so much from my committee and am thankful for their enthusiasm, feedback, and support.

I am so grateful to all of my friends in the Department of Ecology and
Evolutionary Biology and in the BESS GSA. I had an amazing cohort of peers to start my academic journey with who have all provided wisdom, perspective, and laughs at different times. In particular, I have to thank Jenny Uehling, Kara Andres, Lizzie Lombardi, and Lauren Brzozowski for being incredible scientists, athletes, and friends who are always there for a run and a chat. The BESS GSA has been an intellectual energizer throughout my PhD, providing an engaged group of motivated environmental scientists with whom I could share breakthroughs and setbacks, discuss career decisions, and always rely on for honest input on my science. Katie Haviland and Dave Frey were incredible friends and peers who signed on to lead the group with me for two years, for which I am incredibly thankful.

I would never have considered pursuing a PhD without the mentorship and encouragement of my Colgate advisors, Catherine Cardelús and Anthony Chianese. I continue to be inspired by their commitment to and innovation in undergraduate research and scientific teaching. I couldn’t ask for a better start to my scientific career.

Finally, I have to thank my family for their unwavering support of this degree through all of the ups and downs. Thanks to my brother, David, and sister-in-law, Bailey, who allowed me to practice communicating my science and never once complained. To my parents, Dave and Jeanne, who fostered a love of math and science in our family and who will always happily spend dinner time discussing science policy, climate change, and pedagogy. I was privileged to be raised in such a supportive environment and I hope my career has as positive an impact as they have both had on their communities. To my partner, Tom Ryan, for helping me to think through my research when I was stuck and for never doubting my capacity to complete this degree. Also, to my chosen family, whom I would choose all over again: Ditra Backup, Kate Meyer, Phoebe Young, Kristin Andres, Benn Ayd, Steve Day, Elizabeth Evans, Cate Gropper, Molly Gilson, Sus Ivory, and Mabel Baez.
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<td>C</td>
<td>Carbon</td>
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<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
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<tr>
<td>eCO₂</td>
<td>Elevated atmospheric carbon dioxide</td>
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<tr>
<td>FACE</td>
<td>Free-Air Carbon dioxide Enrichment</td>
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<td>IRMS</td>
<td>Isotope Ratio Mass Spectrophotometer</td>
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<td>MAOM</td>
<td>Mineral Associated Organic Matter</td>
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<td>NDFF</td>
<td>Nevada Desert Free-Air Carbon dioxide Enrichment Facility</td>
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<tr>
<td>NPP</td>
<td>Net Primary Productivity</td>
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<td>POM</td>
<td>Particulate Organic Matter</td>
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<td>Py-GC/MS</td>
<td>Pyrolysis- Gas Chromatography/Mass Spectrophotometry</td>
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<td>SIC</td>
<td>Soil Inorganic Carbon</td>
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<td>SOC</td>
<td>Soil Organic Carbon</td>
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<td>SOM</td>
<td>Soil Organic Matter</td>
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CHAPTER ONE

IMPLICATIONS OF CARBONATE REMOVAL METHODS ON A GLOBAL CHANGE STUDY IN THE MOJAVE DESERT
ABSTRACT

One impact of anthropogenic perturbation of the global carbon (C) is changes in global soil carbon stocks. Detecting these changes can be challenging, though, because of the time it for a change in something like elevated atmospheric CO$_2$ to manifest as a change in soil C. Arid ecosystems, which cover 40% of ice-free land globally, are additionally challenging because soil organic C (SOC) concentrations are relatively low while inorganic C concentrations can be high. In order to quantify organic C stocks, acid pretreatment methods are often used to remove inorganic C before analysis of %C. However, acid pretreatments, depending on type and method, have been shown to differentially affect C pools across soils based on characteristics such as the amount and composition of organic matter. Therefore, experiments simulating disturbance such as elevated CO$_2$ may change SOC enough to create within-experiment non-linear effects of acid pretreatments, making comparison between control and treatment soils inaccurate.

Here, we test this theory using two acid pretreatment methods (acid washing with H$_3$PO$_4$ or fumigation with HCl) on soils from the Nevada Desert Free-Air Carbon Dioxide Enrichment Facility (NDFF). We compare the effects of each pretreatment method on SOC and $\delta^{13}$C across CO$_2$ treatments. Further, we use $\delta^{13}$C to explore why the two methods may affect SOC differentially in these soils. We found that acid washing results in a ~36% underestimate of SOC stocks on average and that this organic C loss can differ in control and +CO$_2$ soils. These differences are greatest in soils with higher SOC concentrations but does not always result in differences in +CO$_2$ treatment effects. The two methods result in substantially different $\delta^{13}$C measurements such that with acid washing, soils are more enriched in $\delta^{13}$C and show no +CO$_2$ treatment effects while fumigation results in more depleted $\delta^{13}$C overall and more depleted $\delta^{13}$C in +CO$_2$ soils. These results indicate that current estimates of SOC in the Mojave Desert...
generally may be underestimated and that comparisons of soil analyses from the NDFF experiment may be conflicting if different acid pretreatment methods of SIC removal are used.

**INTRODUCTION**

The Nevada Desert Free Air Carbon Dioxide Facility (NDFF) in the Mojave Desert is the only long-term elevated CO$_2$ experiment in an undisturbed dryland ecosystem. Drylands represent 40% of terrestrial land cover globally (Glenn et al. 1993; Lal 2004) making them highly relevant to global carbon (C) models. Data from NDFF are used to further our understanding of soil ecology and biogeochemistry in deserts (Jin and Evans 2010; Jin et al. 2011; Koyama et al. 2018) and often serve as the sole point representing drylands in metanalyses of ecosystem responses to elevated CO$_2$ (Terrer et al. 2018, 2019, 2021). Accurate quantification of C stocks in this experiment is critical for the correct interpretation of how elevated CO$_2$ will affect dryland C cycling, and the larger patterns of elevated CO$_2$ effects on global C cycling.

Quantifying pools of above and belowground organic C is often a focus of global change studies such as NDFF because these pools are known to respond to anthropogenic disturbances, including changes in temperature, precipitation, or atmospheric CO$_2$ concentration (Norby et al. 2005; Norby and Zak 2011). Stocks of organic C can be challenging to accurately measure and quantify due to spatial and temporal heterogeneity even within a single ecosystem study. In arid ecosystems, directly measuring soil organic C (SOC) is often complicated by the presence of soil inorganic C (SIC). In contrast to organic C, SIC cycles on much longer, geologic time scales of centuries to millennia and is less responsive to short-term perturbations associated with anthropogenic disturbance. Therefore, methods of accurately measuring SOC as distinct from SIC are fundamental to answering questions about changes to the global C cycle and
mechanisms underlying those changes.

Soils at NDFF are Aridisols derived from calcareous alluvium (Jordan et al. 1999) containing high amounts of soil inorganic C (> 90% of total soil C). In order to measure organic carbon stocks in these soils, it is necessary to remove this pool of C prior to direct measurement of SOC (Jordan et al. 1999; Koyama et al. 2019). Pretreatment of soils with inorganic acids removes SIC and leaves behind only SOC that can then be measured directly. An ideal pre-treatment method completely removes SIC without affecting the true organic values of %C, δ^{13}C, and total %N and δ^{15}N. Percent C is needed for the calculation of C stocks, while %N and isotope data provide information that is useful for understanding the mechanism of loss or gain of C. At NDFF, the elevated atmospheric CO_2 treatment used a δ^{13}C depleted source of CO_2 making this pool distinct from atmospheric CO_2. This creates a tracer of newly fixed photosynthate in the ecosystem and the information gained from δ^{13}C_{SOC} measurements provide valuable mechanistic information to support our understanding of how SOC stocks respond to eCO_2.

A number of methods have been developed for removing SIC but many have been shown to affect these values differently based on soil characteristics (Bisutti et al. 2004; Ketchian et al. 2018). Recent studies, including an exhaustive analysis by Brodie et al. (2011b), show non-linear and non-proportional results of SIC removal across methods and across soil types (Fernandes and Krull 2008; Brodie et al. 2011a). In particular, the amount of organic matter, the composition of organic matter, and the form of organic matter stabilization exert some amount of control on the magnitude and direction of how acid affects both the amount and stable isotope ratio of C and N measured (Fernandes and Krull 2008). These non-linear effects make comparisons difficult to interpret if researchers use different methods of SIC pretreatment in experiments with different soil characteristics. What is still not known, however, is if a treatment such as eCO_2 can
affect SOC content and composition enough that using different pretreatment methods on soils from the same experiment could result in different measurements of SOC in soils from the same experiment. Further, soils from within the same experiment may have characteristics that change the effectiveness of a pretreatment method. For example, at NDFF soils were collected from beneath the canopy of five major vegetation cover types and unvegetated interspace because shrubs were expected to respond to eCO$_2$ more directly and therefore have a strong influence on soil organic matter in those microsites (Newingham et al. 2014). The amount of SOC and N in the soil can depend on the size and age of the aboveground shrub vegetation (Schlesinger et al. 1996). These differences in SOC may result in different effects of pretreatment methods on SOC measurements across cover types.

In large-scale, collaborative experiments such as NDFF, multiple labs may analyze soil samples using different acid pretreatment methods. Here, we compare the effects two acid pretreatment methods used on NDFF soils: direct addition of an inorganic acid (“acid washing”) and indirect treatment with volatized acid (“fumigation”) and compare results across control CO$_2$ and eCO$_2$ plots. Acid washing has been used extensively on soils from NDFF (Jin and Evans 2007; Schaeffer et al. 2007; Evans et al. 2014; Koyama et al. 2019). However, this method has been shown to result in organic C and total N loss which could result in under estimated stocks (Fernandes and Krull 2008). To assess this, we compare direct acidification to acid fumigation which is a method that does not involve addition of liquid to the sample and therefore should not suffer from issue. Acid fumigation, though, suffers from other detriments such as potential for incomplete SIC removal and negative impacts on $\delta^{15}$N (Brodie et al. 2011a). The objectives of this study were to 1) Assess how each method affects SOC, total N, $\delta^{13}$C and $\delta^{15}$N relative to each other, 2) Determine if the difference between pretreatment methods affects soils from
control and eCO\textsubscript{2} plots equivalently, and 3) Compare these two methods on a complete set of soils from NDFF to determine if the difference between methods affects conclusions drawn about changes in SOC stocks at NDFF.

We use $\delta^{13}$C of organic and inorganic carbon to understand the impacts of each method on quantifying SOC. Organic C reflects the photosynthetic pathway of organic matter formation during photosynthesis and ranges from -20‰ to -30‰ for C\textsubscript{3} plants and -15‰ to -20‰ for C\textsubscript{4} plants. This isotopic ratio of C in soil is reflective of the source of the organic matter as plant input is incorporated into long-term soil C pools by physical and biological degradation (Smith and Epstein 1971). Soil inorganic C is either from a lithogenic source (marine limestone deposits; $\delta^{13}$C of -2‰ to +2‰) or pedogenic source (dissolution and reformation of lithogenic carbonates; $\delta^{13}$C of -10‰ to 0‰) (Boutton 1991). In a sample with both organic and inorganic C, the $\delta^{13}$C ratio of the soil measured is a linear mix of the $\delta^{13}$C\textsubscript{SOC} and $\delta^{13}$C\textsubscript{SIC}, based on the relative ratio of each pool to the total amount of C. Removal of inorganic C will move the $\delta^{13}$C ratio toward $\delta^{13}$C\textsubscript{SOC} so soil treated with acid will exhibit a more negative (depleted) $\delta^{13}$C relative to the untreated sample. We also measured $\delta^{13}$C of the rinsate resulting from the acid-washing method to determine the amount and nature of the C lost when using this method. Impacts on N are more simple to assess as generally total %N and $\delta^{15}$N pools are quantified and can therefore be compared directly to non-acidified samples. For this detailed analysis of method impacts, we used soil from a focal cover type plant species, \textit{L. tridentata}, which is the largest cover type at NDFF and was shown to respond to eCO\textsubscript{2} with an increase in photosynthesis (Naumburg et al. 2003).

We hypothesized that due to the low clay content of these soils, resulting in high permeability and low aggregation, fumigation would be more effective at SIC removal than acid
washed in NDFF soils. We predict that direct acidification will underestimate SOC and total N stocks and that fumigation will underestimate total N and result in an enriched $\delta^{15}N$ ratio. We further hypothesize that these two methods will differentially affect SOC measurements in soils from ambient and elevated CO$_2$ plots at NDFF, ultimately resulting in different conclusions about the effect of $e$CO$_2$ on SOC stocks in this arid ecosystem. Finally, we predict the difference between pretreatment methods will be inconsistent across soil cover types. To address these hypotheses, we first test each method on soils from the dominant perennial shrub cover type at NDFF, Larrea tridentata. After exploring the impacts on %C, %N, $\delta^{13}C$ and $\delta^{15}N$, we apply both methods to soils from six different cover types at NDFF to see if these differences are consistent across soils experiencing different impacts of elevated CO$_2$.

**METHODS**

Soil samples used in this study were collected in 2007 at the Nevada Desert FACE Facility near Mercury, NV. Cores were taken during the destructive sampling done at the end of this 10-year experiment. We used only the soil from 0 to 20 cm in depth from the surface for this experiment. Soil cores were collected from each of the three elevated (+CO$_2$) and three ambient (control CO$_2$) plots. Within each plot, soils were collected from beneath the canopy of three individuals of five different plant species and from unvegetated interspace for a total of six cover types. This resulted in 18 samples for all analyses completed only on *L. tridentata* soils. When analyzing all six cover types, four samples could not be located in our archive resulting in 104 samples in total. Cores were homogenized, sieved to 2 mm, dried, and stored prior to this analysis. A 20 g sample from each core used was ground to a fine powder using a ball mill.
(8000M Mixer/Mill, SPEX Sample Prep) and all subsequent acid tests were done using subsamples of each 20 g homogenized sample.

**Acid Washing Treatment**

A 1.00 g subsamples of each soil was weighed into 20 mL glass scintillation vial. Four milliliters of 1M H$_3$PO$_4$ acid were added to each vial. After initial effervescence subsided, vials were capped and swirled by hand to ensure all soil was exposed to acid solution. Vial caps were then loosened to allow air flow but left on top of vials to minimize evaporation. Samples were left to settle in a fume hood for 3 hours. Then, the top 1 mL of supernatant acid was pipetted off without disturbing the soil and collected in a glass scintillation vial. This process was repeated twice except that the second and third aliquots were only 2 mL of acid, and after the third addition of acid, samples were left to sit overnight before collecting supernatant. The following day, two milliliters of DI water were added to each vial to remove some acidity from the samples. After 3 hours the supernatant water was then pipetted off and collected in the same glass vial as previously removed acid. This process was repeated twice. After rinsing, soils were dried overnight at 60C and then re-weighed. Soils were broken up and mixed before loading in tins (~20 mg) for dry combustion analysis. In total, ~10-12 mL of rinsate were collected for each sample.

Rinsate solutions were dried in an oven at 60C for three days before analysis by dry combustion, at which point the solution is a highly viscous liquid. In order to get the most accurate weight of the hygroscopic H$_3$PO$_4$ liquid, each sample was left in the oven until immediately prior to weighing the vial for “dry” weight, loading into silver capsules, and run on the IRMS. The amount of carbon in the rinsate was calculated by the weight of the dried rinsate
multiplied by the measured %C of the sample. The resulting amount was then adjusted for the mass of soil (1 g) from which the rinsate was collected to achieve a measure of mg rinsate C per gram of soil. Nitrogen was below detection limits in the acid wash liquid so we are only able to report C value.

_Acid Fumigation Treatment_

Concentrated (12 M) hydrochloric acid was used for acid fumigation due to its high volatility under vacuum. To determine the optimal amount of fumigation time for these soils, we first tested only _L. tridentata_ soil samples at 6, 12, 24 and 48 hours. Previous studies have shown effective carbonate removal after 6 hours for some soil types and that silver capsules begin to disintegrate by 72 hours (Ramnarine et al. 2011). Soils were weighed at 20 mg into open 8x5 mm silver capsules on a microbalance (Sartorius Cubis, Germany) and placed in a 96 well plate. Approximately 10 uL of DI water was added to each 20 mg sample to facilitate permeation of the gas. The uncovered well plate was then placed into a vacuum desiccator along with two open 100 mL beakers of 12 M HCl. The desiccator was sealed with silicon grease to avoid contamination associated with organic sealants (Schubert and Nielsen 2000). The desiccator was placed under vacuum using a hand-held vacuum pump for at least 5 minutes, and then closed. After fumigation, well plates were dried at 60C for 24-48 hours and each sample was wrapped in a tin capsule before dry combustion.

_Comparison of Pretreatment Methods on NDFF Soils_

The two previously described methods of direct (acid washing with 1 M H₃PO₄) and indirect (fumigation with HCl for 24 hours) acidification were used on soils from six cover types
at NDFF. Fumigated samples were weighed directly into silver capsules and analyzed in the same capsule, requiring no adjustment for carbonate mass loss. Acid-washed samples were weighed before and after acidification and drying in order to adjust %C by mass loss. Percent carbon was adjusted by bulk density for 0-20 cm averaged across all plots to obtain SOC stocks (gC * m$^2$).

Table 1.1 Cover type species names and descriptions at the Nevada Desert FACE Facility.

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<tr>
<th>Species</th>
<th>Description</th>
<th>Areal Coverage (%)*</th>
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<tr>
<td><em>Larrea tridentata</em></td>
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<td><em>Lycium andersonii</em></td>
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<td><em>Lycium pallidum</em></td>
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<td>&lt; 1%</td>
</tr>
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<td><em>Ambrosia dumosa</em></td>
<td>Drought-deciduous shrub</td>
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</tr>
<tr>
<td><em>Pleurophis rigida</em></td>
<td>Bunchgrass, N-fixe</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td><em>Interspace</em></td>
<td>No vegetation</td>
<td>~75%</td>
</tr>
</tbody>
</table>

*Areal coverage obtained from USGS long-term vegetation monitoring plot near NDFF (Beatley plot #15- Jackass Flats).

Sample Analysis

All samples were analyzed for $\delta^{13}$C, %C, $\delta^{15}$N, %N using a continuous flow isotope ratio mass spectrometer (IR-MS; Model V Advantage; Thermo Scientific, Bremen, Germany) coupled to an elemental analyzer (Thermo Finnigan Carlo Erba NC2500). Isotope ratios are expressed as $\delta$ values (per mil):

$$\delta^{13}\text{C}=\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right) \times 1000(\%e)$$

where $R_{\text{sample}}$ and $R_{\text{standard}}$ are the ratios of heavy to light isotope of the sample relative to the international standards for C and N, Vienna-Pee-Dee Belemnite and atmospheric N$_2$, respectively. All isotopic analyses were completed at the Cornell University Stable Isotope
Laboratory (COIL). Percent C was first adjusted to concentration (mg C • g soil⁻¹) and then to stocks (g C • m²) by multiplying by bulk density (g soil * m⁻²). Bulk density was obtained for the 0-20 cm depth of the soil profile in kg soil • ha⁻¹ per plot and then averaged across all plots.

Statistical analyses

All statistical analyses were conducted using the statistics package R, version 4.1.0 (http://cran.r-project.org). All response variables for *L. tridentata* soils (mg C • g soil⁻¹, mg N • g soil⁻¹, δ¹³C, δ¹⁵N) were analyzed using linear mixed-effect models. The effects of increasing time of fumigation on *L. tridentata* soil was analyzed with “CO₂ treatment” and “hours” as fixed effects and “plot” as a random effect. Acid washing effects on *L. tridentata* soils were analyzed with “CO₂ treatment” as the only fixed effect for %C and %N, “CO₂ treatment” and “C source” (soil or acid rinsate) and their interaction for δ¹³C, and “plot” as a random effect for both models.

To compare these two methods of acid pretreatment methods on *L. tridentata*, all responses were analyzed using “CO₂ treatment” and “method” and their interaction as fixed effects and “plot” as a random effect.

To assess the effect of pretreatment methods on eCO₂ effects at NDFF, SOC stocks (g C • m⁻²) and δ¹³C from six cover types with “CO₂ treatment”, “method”, “cover type” and all interactions as fixed effects and “plot” and “sample ID” as a random effects. Residuals were inspected to assess normality and where data did not meet assumptions of normality required for parametric tests, values were log or square root transformed. The relationship of SOC stocks and δ¹³C between pretreatment methods was determined using simple linear regression across all soil samples (n = 104). Distribution of residuals was examined to assess fit. Type III test of fixed effects were assessed with Satterthwaite approximation of degrees of freedom using the
“lmerTest” package (Kuznetsova et al. 2017). Post-hoc pairwise comparisons were conducted using the R package “emmeans” (Lenth 2020). Significance was set at alpha = 0.05.

RESULTS

Fumigation was rapidly effective, removing the majority of SIC within 6 hours (Fig 1.1). This is indicated by a significant drop in the amount of carbon from 26.5 mg C•g soil\(^{-1}\) to 3.48 mg C•g soil\(^{-1}\) (-87%) for soils from control CO\(_2\) plots and from 24.2 to 3.33 mg C•g soil\(^{-1}\) (-86%) for soils from elevated CO\(_2\) plots. The loss of SIC was also supported by a shift in \(\delta^{13}C\) from inorganic (control = -4.7‰ and +CO\(_2\)=-3.8‰) to more organic values (control = -24.23‰ and +CO\(_2\)= -24.9‰) (Fig 1.1). There were not main effects of time, CO\(_2\) treatment, or time*CO\(_2\) treatment on SOC concentration (mg C•g soil\(^{-1}\)) among the four fumigation time points tested (p > 0.05). However, there was a significant effect of time on \(\delta^{13}C\) among the four times (F = 6.6, p < 0.001). Pairwise comparisons showed that fumigating for 48 hours results in a significantly more depleted \(\delta^{13}C\) than at 6 hours for both control and +CO\(_2\) treatments (t= 2.65, p =0.048), although the difference is less than 1‰ across all time points.

Acid washing with H\(_3\)PO\(_4\) resulted in a decrease in SOC concentration from 26.6 mg C•g soil\(^{-1}\) prior to acidification to 3.4 mg C•g soil\(^{-1}\) for the control CO\(_2\) samples, or a decrease of 87%. Soils from the elevated CO\(_2\) treatment showed a similar loss of carbon, from 24.2 mg C•g soil\(^{-1}\) to 2.52 mg C•g soil\(^{-1}\), a 90% decrease. The difference in SOC concentrations between CO\(_2\) treatments after acidification was not different (t= 1.16, p=0.31) but there was a difference of -0.57‰ in \(\delta^{13}C_{SOC}\) between control and +CO\(_2\) treatment (t=2.28, p= 0.04) (Fig 1.2). We also analyzed the amount of C in the collected acid and water that results from the wash method and recovered approximately 6% of SOC from both CO\(_2\) treatments. \(\delta^{13}C\) of the collected rinsate
indicates that the C is organic and shows a difference of -0.59‰ between CO$_2$ treatments (t=2.38, p=0.03). The organic C removed in the washing process has a slightly different composition than the remaining soil, on average being about 1‰ more enriched (t= 3.8, p < 0.001).

Figure 1.1 Effect of increasing fumigation time on SOC concentrations and δ$^{13}$C in _L. tridentata_ soils. There is a decrease in SOC concentration after 6 hours but no further changes over 48 hours. SOC- δ$^{13}$C also decreases after 6 hours, then remains constant until a decrease in δ$^{13}$C at 48 hours. There were no differences between CO$_2$ treatments. n = 9 for each CO$_2$ treatment.
Fig 1.2 Organic C measured in soil and rinsate after pretreatment with acid washing method. There is no difference in SOC in either soil or rinsate across CO$_2$ treatments but $\delta^{13}$C is more depleted in soils than rinsate for both control and +CO$_2$ samples. Whiskers in bar plots are standard error. Asterisk indicated significant difference between CO$_2$ treatments (pairwise comparison, $p < 0.05$).
Direct comparison of fumigation and acid washing on SOC concentration and $\delta^{13}\text{C}_{\text{SOC}}$ showed a substantial difference between methods in SOC concentration (mg C•g soil$^{-1}$) ($F=7.96$, $p = 0.008$) and $\delta^{13}\text{C}_{\text{SOC}}$ ($F=24.8$, $p < 0.001$). Neither method of acidification shows a CO$_2$ treatment effect on SOC concentration ($F= 1.78$, $p = 0.25$) but both methods show a decrease in $\delta^{13}$C under elevated CO$_2$ ($F = 9.27$, $p = 0.004$). There is no difference in the CO$_2$ treatment between methods for SOC concentration (method* treatment, $F= 0.15$, $p = 0.70$) or $\delta^{13}$C ($F = 1.5$, $0.23$) despite the effect of +CO$_2$ on $\delta^{13}$C with fumigation (-1.3‰) being more than twice as large as the difference from acid washing (-0.57‰).

**Fig 1.3** Comparisons of A) soil organic carbon concentration (mg C•g soil$^{-1}$) and B) $\delta^{13}$C of *L. tridentata* soils using two different acid pretreatment methods. Fumigation yields higher SOC concentrations and more depleted $\delta^{13}$C relative to acid washed soils across both CO2 treatments. Whiskers in bar plots are standard error. Asterisk indicated significant difference between pretreatment methods (pairwise comparison, $p < 0.05$).
The effect of acidification on nitrogen (N) can be compared directly to non-acidified values because there is no need to remove inorganic N; post-acidification N is ideally the same as non-acidified N. When comparing fumigation and acid wash methods to non-acidified soil N concentration (mg N•g soil\(^{-1}\)), there was a main effect of methods (F= 6.88, p = 0.002) but no main effect of CO\(_2\) treatment (F= 1.49, p = 0.29) or method*treatment (F = 0.18, p = 0.82) (Fig 4). The acid washing method results in a loss of ~0.1 mg N•g soil\(^{-1}\), which is 30% of control CO\(_2\) total N and 24% of + CO\(_2\) total N, neither acid washing or fumigation resulted in different amounts of total soil N from pre-acidified values. The only difference across methods was between fumigation and acid washing, where fumigation results in higher concentrations of N for +CO\(_2\) treatment than the same samples subjected to acid washing (t= -2.29, p= 0.015). This difference, however, does not affect the CO\(_2\) treatment effect on N concentration, which was non-significant for all methods.
The response of δ¹⁵N to acidification methods closely matched that of N concentrations. There were differences across methods (F= 132, p < 0.001), but no main effect of +CO₂ treatment (F= 2.16, p= 0.21) or method*treatment effect (F = 1.02, p = 0.37). Pairwise comparisons show that for soils from both control and +CO₂ plots, fumigation results in more
depleted $\delta^{15}$N relative to non-acidified samples ($t=-8.8$, $p<0.001$) and acid washing results in more enriched $\delta^{15}$N ($t=2.99$, $p=0.012$). Regardless, these differences in absolute $\delta^{15}$N values did not change the CO$_2$ treatment effect, which, like N concentration, was non-significant for all methods.

*Effects of pretreatment on NDFF Soil Organic Carbon*

SOC stocks were linearly related between pretreatment methods (linear regression, $\text{SOC}_{\text{washing}} = \text{SOC}_{\text{fumigation}} * 0.92 + 215.5$, $R^2 = 0.62$, $p < 0.001$, Fig 1.5A). This result indicates that acid washing underestimates SOC concentrations relative to fumigation across NDFF soils and that this difference increases with SOC concentration (i.e. samples with higher SOC concentrations result in greater loss in the washing process). The difference in SOC stock measurements between pretreatment methods had significant main effect of cover type ($F=7.79$, $p<0.001$), no main effect of CO$_2$ treatment ($F=0.94$, $p=0.33$), but an interaction between CO$_2$ treatment and cover type ($F=6.0$, $p<0.001$). *Larrea tridentata* and *L. andersonii* soils had a larger difference between pretreatment methods for control CO$_2$ soils than for eCO$_2$ soils where acid washing underestimated SOC stocks more in control soils (Fig 1.6). *Lycium pallidum* showed the opposite trend, with a greater difference between pretreatment methods for eCO$_2$ soils. In general, there was a trend of soils with lower SOC stocks having smaller difference between fumigation and acid washing pretreatments.
Fig 1.5 Linear relationship between soil organic carbon A) concentration and B) δ¹³C measured after acid washing pretreatment and fumigation pretreatment in soils collected from Nevada Desert FACE facility. Solid line represents 1:1 relationship, which would indicate that pretreatment methods produce the same results. Samples are indicated as being from control or +CO₂ plots by shape. P and R² values are derived from linear regression.
CO₂ treatment had only a marginal effect on SOC stocks (g C • g m⁻²) when compared across pretreatment methods, indicating that overall SOC stocks did not exhibit a strong response to the experimental treatment (eCO₂) regardless of the acid pretreatment method used (F = 6.11, p = 0.069; table 2; Fig 1.7). However, there were main effects of acid pre-treatment method (F= 122.8, p < 0.001) and plant cover type (F= 34.82, p < 0.001) and an interactive effect of CO₂.
Carbon stocks are on average 20% larger when measured by fumigation rather than direct acidification. This difference is +20% control CO₂ soils and +29% in +CO₂ soils. Pairwise comparisons show that CO₂ treatment effects were consistent across most cover types. Interspace, *P. rigida*, *A. dumosa*, and *L. pallidum* do not show a change in SOC stock under eCO₂ when measured by either pretreatment method (Table 1.2). *Lycium andersonii* shows a significant loss of SOC under eCO₂ for both washing (t= 2.93, p = 0.004) and fumigation (t= 2.91, p = 0.004). *Larrea tridentata* exhibits a marginal loss of SOC under eCO₂ with acid washing (t= 1.95, p = 0.053) and no change in SOC with fumigation (t= 1.39, p = 0.16).

![Fig 1.7 Comparison of SOC stocks (g C m⁻²) between soils from control and +CO₂ plots at the Nevada Desert FACE Facility. Panels represent two different methods of acid pretreatment applied to soils. Asterisk indicates significant difference between CO₂ treatments (pairwise comparison, *= p < 0.05, **= p < 0.01).](image)
Table 1.2 Fixed effects in a mixed-effects linear model for SOC stocks (g C • g m⁻²). “Method” refers to acid pretreatment method (fumigation or washing) and “Treatment” to atmospheric CO₂ (control or elevated). Stocks were square root transformed for normality prior to analysis. Plot and sample ID were included as random effects. n = 104.

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<th>Variable</th>
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<th>Prob &gt;F</th>
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<td>Treatment<em>Method</em>Cover Type</td>
<td>0.21</td>
<td>0.96</td>
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Fig 1.8 Comparison of δ¹³C (‰) between soils from control and +CO₂ plots at the Nevada Desert FACE Facility. Panels represent two different methods of acid pretreatment applied to soils. Asterisk indicates significant difference between CO₂ treatments (pairwise comparison, *= p < 0.05, ** = p < 0.01).
Unlike SOC stocks, there was no relationship in \( \delta^{13}C \) measurements between pretreatment methods \((t = 1.59, p = 0.114)\) (Fig 5B). \( \delta^{13}C \) of SOC varied by method \((F = 332.0, p < 0.001)\), cover type \((F = 11.7, p < 0.001)\), and marginally by CO\(_2\) treatment \((F = 6.7, p = 0.07)\). There were also several interactive effects among these variables (Table S2). There was no effect of CO\(_2\) treatment for \( \delta^{13}C \) in soils from any cover type when soils were pretreated with acid washing (Fig x). Soil pretreated by fumigation showed greater variation in \( \delta^{13}C \) across cover types and by CO\(_2\) treatment. Specifically, soils from *P. rigida* \((t = 5.3, p < 0.001)\), *L. andersonii* \((t = 4.17, p < 0.001)\), and *L. tridentata* \((t = 2.08, p = 0.04)\) all exhibited depleted \( \delta^{13}C \) ratio under eCO\(_2\) treatment. *Ambrosia dumosa* soils showed an unexpected enrichment in \( \delta^{13}C \) under eCO\(_2\) \((t = -2.14, p = 0.04)\) while interspace and *L. pallidum* soils showed no treatment effects. Soils pretreated with fumigation had on average a \( \delta^{13}C \) ratio 3.2‰ more depleted than acid washed soils.

Table 1.3 Fixed effects in a mixed-effects linear model for SOC \( \delta^{13}C \) (‰). “Method” refers to acid pretreatment method (fumigation or washing) and “Treatment” to atmospheric CO\(_2\) (control or elevated). Plot and sample ID were included as random effects. \( n = 104 \).

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<th>Variable</th>
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DISCUSSION

We found that fumigation is a more effective method of removing SIC from NDFF soils when quantifying SOC concentrations than the commonly used acid washing method. Soils treated with fumigation retain more SOC and less SIC as identified by the higher %C and more organic δ^{13}C ratio of treated samples (Fig 3). Soils treated with acid washing have lower SOC concentrations due to organic matter loss during the rinsing process (Fernandes and Krull 2008). We demonstrated this source of C loss by collecting the rinsate produced during acid washing, quantifying the amount of C in this liquid, and identifying it as organic by its δ^{13}C ratio (Fig 2).

We also confirmed that %N and δ^{15}N should be measured in a separate set of non-acidified samples as fumigation depletes δ^{15}N measurements and acid washing results in a loss of ~25% of total N (Fig 4).

Fumigation removed the majority of inorganic C from *L. tridentata* soils in as little as 6 hours as evidenced by a decrease of 88% of total C and a shift in δ^{13}C toward organic values (Fig 1). We attribute the effectiveness of this method to the low clay content of these soils (average of 2-3% clay, < 3 µm), which results in minimal aggregation and high porosity for maximum surface area reaction of HCl gas with SIC. Increasing fumigation time has little effect on C concentration and δ^{13}C after 6 hours except for a decrease in δ^{13}C at the 48-hour time point. We attribute this depletion of δ^{13}C to degradation of the silver capsules, resulting in a small amount of organic matter loss. This loss is not enough to substantially change the SOC concentration, but enough to decrease δ^{13}C by about 1‰, indicating a potential fractionation between the organic matter lost and what remains. Based on the results of this trial, we chose to fumigate all other samples in this study for 24 hours to ensure maximum reaction of the soil without risking a loss of SOC.
Acid washing with 1M H$_3$PO$_4$ removes most SIC in *L. tridentata* soils, with a 90% decrease in measured C concentration and a depletion in $\delta^{13}$C toward organic values (Fig 2). However, relative to SOC measured by fumigation, acid washing results in 24% less SOC in control CO$_2$ samples and 30% less SOC in +CO$_2$ samples (Fig 3). It is important to note that while the absolute loss of about 1.0 mg C$\cdot$g soil$^{-1}$ is a small amount for most soils, in the Mojave Desert this can represent more than 25% of total SOC for soils under *L. tridentata*. The loss of SOC due to acid washing may be insignificant in ecosystems where SOC values are higher, but this represents a significant source of error in quantifying SOC stocks at NDFF. Acid washed samples are also slightly enriched in $\delta^{13}$C relative to fumigated soils, which could be attributable to incomplete SIC removal. Even a small amount of SIC remaining can significantly impact $\delta^{13}$C because $\delta^{13}$C of SIC is much more enriched (-2‰ to +2‰) than SOC. Overall, acid washing results in underestimation of SOC stocks and inaccurate $\delta^{13}$C data likely due to SIC retention.

In addition to quantifying the differences in pretreatment methods on SOC, we also considered the impacts on total N measurements (Fig 4). It has been documented several times that pretreatment of soils with acid affects total nitrogen (N) and $\delta^{15}$N adversely (Fernandes and Krull 2008; Brodie et al. 2011b), however, measuring C and N on one sample is highly desirable to decrease time and cost of sample analysis and so worth exploring. Our results confirm earlier studies that N can be underestimated due to loss of organic compounds in the acid washing method of pretreatment. Although the absolute loss was small (0.1 mg N$\cdot$g soil$^{-1}$), the relative loss was 24% of total N for soils from control CO$_2$ plots and 30% from +CO$_2$ plots. This parallels the difference in SOC between fumigation and acid washing across CO$_2$ treatments. Selective solubilization of compounds during acid washing can have varying impact on $\delta^{15}$N based on the composition of organic matter (Fernandes and Krull 2008). Our results confirm that
acid washing impacts δ^{15}N, with consistent effects across CO\textsubscript{2} treatments (+1.37‰ for control CO\textsubscript{2} soils and +1.74‰ for + CO\textsubscript{2} soils). Pretreatment with fumigation results more accurate N concentration measurements as determined by comparison to %N of non-acidified samples. However, fumigation results in depleted δ^{15}N possibly due to volatization of δ^{15}N enriched compounds. These results confirm that it is best practice to obtain N data by running non-acidified soils in parallel with acidified soils.

Effects of Acid Pretreatment on NDFF Soils

Direct comparison of acid pretreatment methods on NDFF soils showed substantial variation in SOC stocks and δ^{13}C measurements across cover types and CO\textsubscript{2} treatment. The largest difference between acid washing and fumigation is the magnitude of carbon stocks measured; acid washing on average underestimates SOC stocks by 39% relative to fumigation in NDFF soils. The difference in SOC stocks between methods is smallest at low SOC concentrations and greatest in samples with high SOC concentration (Fig 1.5A). This is consistent with our hypothesis that soil content and composition may cause differences in how these pretreatment methods affect SOC measurements.

Two pools of SOC are susceptible to loss during acid washing. The first is light particulate organic matter that is less dense than the liquid used during acidification. This pool of SOC is decanted with the acid and water when it separates from the denser soil fraction. Grinding samples prior to analysis may minimize this loss but will not remove it entirely. The second pool of C that is susceptible to acid washing is organic matter stabilized by Ca\textsuperscript{2+} bridging or physically occluded by carbonate deposition (Bronick and Lal 2005; Virto et al. 2018). These stabilization mechanisms have recently been recognized as important to SOC stabilization in
basic soils (Rowley et al. 2018). The decrease in pH when H₃PO₄ is added to the soils disrupts these mechanisms and increases the pool of SOC vulnerable to loss. Acidification via fumigation disrupts these same stabilization mechanisms but the organic matter is retained in the capsule for analysis and so there is no loss of SOC.

The difference in SOC stocks measured by these pretreatment methods varied by cover type and CO₂ treatment (Fig 1.6). Samples with the highest SOC concentrations are from control CO₂ plots, driving the interaction between pretreatment method and CO₂ treatment. Cover types with low SOC concentrations generally showed the smallest difference between acid washing and fumigation. For these cover types (interspace, P. rigida, and A. dumosa) there was also no difference in how pretreatment method affected SOC measurements across CO₂ treatments. In contrast to this, soils with higher SOC concentration had substantial differences in SOC measurements by CO₂ treatment. Lycium andersonii and L. tridentata soils from control CO₂ plots when treated with acid washing exhibited substantial underestimates of SOC stocks (-282 ± 31 and -343 ± 43 g C•m⁻², respectively). Soils from these cover type in eCO₂ plots, though, showed smaller differences between fumigation and acid washing. The response of Lycium pallidum soils to pretreatment method exhibited the opposite trend, with a greater underestimate of SOC in eCO₂ soils with acid washing (+291 ± 34 g C•m⁻²) relative to control CO₂ soils (157.8 ± 23.7 g C•m⁻²).

Ultimately, our understanding of how eCO₂ affects SOC stocks at NDFF is impacted by the differences between pretreatment methods for SIC removal. Using fumigation, L. andersonii is the only cover type where soils exhibit a change in SOC stocks under eCO₂ (-255.4 ± 61.9 g C•m⁻², p = 0.004) (Fig 7B). Acid washing pretreatment shows a similar, although smaller, decrease in SOC for L. andersonii (-150.6 ± 36.3 g C•m⁻², p = 0.004) in addition to a loss of
SOC for *L. tridentata* soils (-153.0 ± 75.6, p = 0.049) (Fig 1.7). This is an important difference because *L. tridentata* is the largest vegetation cover type in the Mojave Desert, covering approximately ~12% of surface area at NDFF (Jordan et al. 1999). In some studies of NDFF soils, *L. tridentata* and interspace are the only two cover types measured as end points for how this desert will respond to eCO₂ or only *L. tridentata* is studied because it had the largest response to eCO₂ (Jin and Evans 2007, 2010; Jin et al. 2011). The pretreatment method chosen in this case could affect the reported results and possibly interpretation of other parts of these experiments.

Carbon stable isotope data are a valuable part of the NDFF experiment and can help explain how SOC stocks change under eCO₂. We found substantial differences in how pretreatment methods affect δ¹³C in these soils. There is not a consistent linear bias in δ¹³C measurements between fumigation and acid washing (Fig 5B) as there is with SOC. δ¹³C measurements in acid washing generally results in more enriched δ¹³C ratios (mean = –22.8 ± 0.17‰) than fumigation (-26.0 ± 0.17‰) and there is also a greater range of δ¹³C represented in soils that are fumigated (-32‰ to -22‰) relative to acid washing (-19‰ to -25‰). SOC with a more depleted δ¹³C ratio is likely newer, less microbially processed C and so has a δ¹³C closer to fresh plant matter. This could indicate that this pool of C is lost during the rinsing process of acid washing. This difference between methods could be also due to incomplete removal of SIC which is more δ¹³C enriched. Critically, when soils are acid washed there are no differences in δ¹³C for any cover type but when soils are pretreated with fumigation four cover types show differences in δ¹³C by CO₂ treatment (Fig 1.8). Previous analysis of these samples used δ¹³C and a mass balance to quantify the amount of C from above (70%) and belowground (30%) sources (Evans et al. 2014). This information can inform our understanding the mechanisms of C gain or
loss under elevated CO$_2$, but the conclusions are only as accurate as the data from which they are drawn. Pretreatment of soils with acid washing gives an inaccurate picture of $\delta^{13}$C of SOC and analysis of these soils with fumigation would result in significantly different conclusions about C cycling under elevated CO$_2$ at NDFF.

We believe the differences in how acid pretreatment differentially affects SOC stocks in control and elevated CO$_2$ plots may be reflective of a change in the composition of SOC. Elevated CO$_2$ can change SOC stocks directly by increasing the amount of C added to the soil or indirectly via changes in microbial activity and carbon use efficiency (Carney et al. 2007; Schimel and Schaeffer 2012; Sulman et al. 2014; Schimel et al. 2015). The composition and stability of SOC stocks is dependent on the type of organic C entering the soil (i.e. plant litter or low molecular weight compounds) and where C is entering the soil matrix (above- or belowground) (Kallenbach et al. 2015; Sokol and Bradford 2019; Lavallee et al. 2020). These changes may impact the amount of C that is susceptible to loss during acid washing. For example, an increase in the amount of litter produced or the rate at which litter is decomposed under eCO$_2$ can alter the pool of light particulate organic matter (Cardon et al. 2001; Dorodnikov et al. 2011). In NDFF soils, a decrease in particulate organic matter under eCO$_2$ would be reflected as the smaller difference between acid washing and fumigation in eCO$_2$ soils.

Elevated CO$_2$ also changes SOC stabilization mechanisms such as aggregation structure or mineral associations (Six et al. 2001; Henry et al. 2005; Dorodnikov et al. 2011; Hofmockel et al. 2011). Soil organic matter stabilization by calcium (Ca$^{2+}$) bridging and carbonate deposition may be prominent in basic soils but are susceptible to disruption when soils are acidified (Lal et al. 1999; Bronick and Lal 2005; Rowley et al. 2018). Plants can alter the composition of root exudation in order to affect the pH of the surrounding soil to solubilize phosphate complexes or
alter the microbial community (Hinsinger et al. 2003; Khorassani et al. 2011; Lareen et al. 2016). Root exudates composition can consist of organic acids, amino acids, and sugars, among other compounds (Hinsinger et al. 2003) and can drive belowground ecosystem responses to eCO$_2$ (Phillips 2007; Phillips et al. 2011). Increasing acidic root exudate flux may destabilize organic matter that is protected from microbial access by carbonate structures and calcium mediated organo-mineral associations (Fig 1.9). Destabilization and mineralization of SOC released due to a change in pH could explain the trend of lower SOC concentrations under eCO$_2$ and also the decrease in SOC lost during acid washing in eCO$_2$ soils. While we cannot test this hypothesis directly with this study, it would be valuable to further understand how acidification is related to change in SOC under eCO$_2$ in basic soils.
Fig 1.9 A) Under control CO₂ conditions, some SOC in NDFF soils is protected by calcium bridge mineral association and in pedogenic carbonate structures. This protects C from microbial decomposition. B) When soils from control CO₂ conditions are pretreated for inorganic C removal, acidification destabilized cation bridging and carbonate structures. Acid washing results in loss of light particulate organic matter and the acid instable pool of C. Fumigation retains these pools resulting in higher SOC measurements relative to acid washing. C) Under elevated CO₂ root exudates result in destabilization of cation bridging and carbonate structures, resulting in microbial mineralization of this C. Addition of root exudates may also stimulate decomposition of particulate or mineral associated organic matter. D) Loss of SOC under eCO₂ due to addition of root exudates decreases the amount of C lost during acid washing and reduces the difference in SOC measurements between fumigation and washing pretreatment methods.
CONCLUSION

There are relatively few studies of arid ecosystem soil C stocks and inaccurate measurements of these stocks could have significant implications for models of global C cycles. The presence of inorganic C in arid soils and the methods used to remove it complicates organic C measurements and requires careful consideration as to how soils are treated. In our analysis of soils from the Nevada Desert FACE facility, two commonly used methods of acid pretreatment produce differing effects of elevated atmospheric CO\textsubscript{2} on SOC stocks and δ\textsuperscript{13}C. Most significantly, pretreatment with acid washing results in SOC stocks that are on average 39% smaller than when pretreated with the acid fumigation method. The amount of SOC lost during acid washing can be non-proportional across soils from control and eCO\textsubscript{2} plots, depending on the vegetation cover. In some cases, the loss of SOC during acid washing leads to different conclusions about how eCO\textsubscript{2} will affect stocks. The loss of SOC during the acid washing process can be a relatively small amount (~1.0 mg C•g soil) but in dryland soils, that amount of C is a substantial fraction of the total SOC pool. Pretreatment methods are also impactful on δ\textsuperscript{13}C measurements because very small losses of SOC or any small amount of remaining SIC can dramatically impact δ\textsuperscript{13}C. This study highlights the potential for changes in soil organic matter caused by an experimental treatment to affect the methods used in analyzing those samples. It is critical to understand these potential methodological biases within and across experiments when interpreting data from global change studies.
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CHAPTER TWO

EFFECTS OF ELEVATED ATMOSPHERIC CO2 ON SOIL ORGANIC CARBON IN THE
MOJAVE DESERT: A TALE OF SMALL QUANTITIES, STATISTICAL ANALYSES, AND
INORGANIC CARBON REMOVAL
ABSTRACT

Soils are a major component of the global carbon cycle, yet our understanding of how soil carbon stocks respond to global change remains limited. Predicting the effects of elevated atmospheric CO$_2$ (eCO$_2$) on soil organic carbon (SOC) is critical to understanding the future global carbon cycle. Arid ecosystems have large spatial coverage globally and are known to be a critical component of the global land sink on an interannual scale, implying that arid soils are important to C cycling under future climate scenarios. However, because of low overall organic carbon content, high spatial heterogeneity and the presence of inorganic carbon, measuring SOC in these environments is challenging.

As an example, the intensively studied Nevada Desert Free-Air CO$_2$ Enrichment Facility (NDFF) reports conflicting results regarding the effects of eCO$_2$ on SOC. Here, we present measurements of SOC stocks from this site and compare the results to the most prominent previous analysis of SOC stocks from the site. We found that the eCO$_2$ treatment resulted in losses of SOC stocks under the major plant cover type in this ecosystem. When scaled to the ecosystem level, however, SOC stocks exhibited no change due to a lack of treatment effect on all other cover types. This contrasts with the previous analysis of these soils showing a 20% increase in ecosystem SOC stocks (1). In an attempt to reconcile these opposing treatment effects, we compare statistical models and inorganic carbon removal methods used in each study. Statistical approaches and inorganic C removal methods did impact ecosystem SOC estimates, but neither completely explain the opposing ecosystem-level treatment effects. Resolving this discrepancy is critical to our understanding of C cycling in global drylands under future climate change scenarios.
INTRODUCTION

Anthropogenic increase of atmospheric CO\textsubscript{2} is expected to have widespread perturbations on global carbon cycling (Kimball et al. 1993, p. 2; Friedlingstein et al. 1995; Cox et al. 2000, p. 2; Friend et al. 2014; Schimel et al. 2015). In order to understand the implications of this disturbance, we must be able to accurately measure changes in global carbon stocks. Terrestrial ecosystems are one of the largest pools of organic C, storing 450-650 Gt carbon as plant biomass and three times as much, 1500-2400 Gt of carbon, as soil organic matter (Friedlingstein et al. 2019). Elevated atmospheric CO\textsubscript{2} (eCO\textsubscript{2}) has been demonstrated to affect SOC stocks by increasing rates of photosynthesis, which can then result in greater amounts of organic carbon inputs to the soil. The increase in net primary productivity (NPP) caused by eCO\textsubscript{2} has the potential to sequester carbon if newly added carbon is allocated to stable pools of soil organic matter (Schimel 1995; Hungate et al. 2009).

Much of the uncertainty in global carbon stocks is attributed to the high spatial heterogeneity of the soil environment, making changes challenging to measure and less frequently quantified compared to aboveground biomass (Jobbágy and Jackson 2000; Jackson et al. 2017; Friedlingstein et al. 2019). Despite this, measuring belowground pools of carbon, including soil organic carbon and roots, is critical to understanding changes in overall ecosystem carbon stocks. In some ecosystems, changes in belowground carbon may constitute the main response to eCO\textsubscript{2} (Kuzyakov et al. 2019; Hu et al. 2020; Castañeda-Gómez et al. 2020). In these instances, failure to accurately measure the belowground changes in carbon stocks does not capture the overall impact of eCO\textsubscript{2} on the ecosystem. Therefore, accurate measurements of SOC are a critical component of understanding carbon cycling under future climate change.

Arid ecosystems cover 40% of Earth’s terrestrial surface and are particularly sensitive to the impacts of global change (Lal 2004). The NDFF experiment was started in 1997 to
understand the long-term impacts of eCO$_2$ on intact desert ecosystems. During 10 years of exposure to 550 ppm atmospheric CO$_2$, increases in photosynthesis rates and biomass growth were observed only in years with high precipitation (Naumburg et al. 2003b; Housman et al. 2006) and this short-term response to eCO$_2$ did not translate into long-term biomass accumulation over the course of the experiment (Newingham et al. 2013). Transitory increases in soil respiration without changes in root growth suggested an increase in heterotrophic activity under eCO$_2$ (de Soyza et al. 2005; Phillips et al. 2006).

The ensuing analyses of SOC from the NDFF presented results showing everything from a 20% increase in SOC stocks across the whole ecosystem, to another showing increases in SOC limited to specific plant cover types, and yet another suggesting no change in SOC (Evans et al. 2014; Koyama et al. 2018, 2019). These contrasting results are an indication of the challenges of quantifying SOC in arid ecosystems. In this work, we present a fourth analysis of SOC at this site and focus on comparing these new estimates to those presented in Evans et al. (2014) that analyzed SOC stocks at a cover type and ecosystem level. This particular data set is important to address directly because it has been used in several reviews and meta-analyses attempting to infer mechanisms of SOC change under eCO$_2$ (Terrer et al. 2018, 2021; Lal 2019; Walker et al. 2020).

Through this direct comparison we identify two issues that stand out as potential sources of difference and both are specific to desert ecosystems. First, the application of appropriate statistical models for analyzing data collected from spatially heterogeneous deserts. And two, the analytical methods used to isolate pools of organic carbon in soils dominated by inorganic carbon. Each of these issues individually has the potential to produce different SOC estimates, and in combination, may completely obfuscate eCO$_2$ experimental treatment effects. We
rigorously test the impacts of the two potential sources of error to understand how soils from the same experiment can yield opposite eCO$_2$ treatment effects.

*Statistical Analysis Considerations*

Understanding the ecology of a study system is critical to designing an appropriate statistical model for data analysis. The Mojave Desert exhibits a high degree of spatial heterogeneity across the ecosystem due to the distinct biogeochemistry associated with plant distribution patterns (Schlesinger et al. 1996). Arid ecosystem plants widely due to limited resources, primarily water, leaving a significant amount of unvegetated space relative to more mesic systems (Schlesinger and Pilmanis 1998). Plants are adapted to capture as much precipitation as possible and the moisture captured by plant canopies is cycled tightly within the immediate vicinity, creating “islands of fertility” of higher carbon and nutrient concentration underneath the canopy of each individual plant (Schlesinger et al. 1990; Schlesinger and Pilmanis 1998; Schimel 2010). Individuals of larger and more long-lived plant species have a greater “island effect” due to their capacity to trap more water and produce more biomass, creating a positive feedback loop of growth and resource retention. These ecological considerations are important when modeling data from the NDFF because different cover types, and even different individuals of the same species, may have distinct SOC responses to eCO$_2$. Using a statistical model capable of differentiating treatment effects among cover types gives important spatial resolution to the treatment effect on the overall ecosystem carbon stock.

*Treatment of Soil Inorganic Carbon*

Quantifying the total amount of carbon in soil using elemental analysis is a basic and
highly accurate measurement, but the method suffers from an inability to differentiate between organic and inorganic carbon. These two forms of carbon cycle on very different time scales; SOC is more responsive to eCO$_2$ as the size of the SOC pool is controlled primarily by the input of plant biomass and decomposition by the soil microbial community, while soil inorganic carbon (SIC) cycling is primarily a geologic process (Chapin et al. 2011). Therefore, measuring organic carbon is the primary focus of global change studies such as FACE experiments. Soils at the Mojave Desert FACE are characterized by high amounts of SIC (> 2%) and low amounts of SOC (< 0.2%) (Jordan et al. 1999). To get an accurate measurement of SOC when using elemental analysis, it is necessary to first fully remove the inorganic carbon. The most common way to remove SIC is to use strong acids such as HCl and H$_3$PO$_4$ to react with CaCO$_3$, resulting in the off gassing of inorganic carbon as CO$_2$. However, these methods have been documented to affect %C and $\delta^{13}$C of SOC (Midwood and Boutton 1998; Fernandes and Krull 2008). The extremely low SOC concentrations in these soils make these effects problematic as even small losses of SOC, that might be considered negligible in soils with more organic matter, have the potential to affect the accuracy of SOC stock measurements (Jensen & Sparks, in prep).

In this study, we present a new analysis of SOC at the Mojave FACE site with subsamples of the same soils used in previously published analyses but using alternative statistical analysis and carbonate removal methods. Using this alternative approach, we found a loss of SOC for the largest plant cover type in the Mojave, *Larrea tridentata*, but no eCO$_2$ treatment effect on SOC stocks when scaled to the ecosystem level using areal coverage of multiple cover types. After presenting the results of this new analysis of SOC from the NDFF, we test two hypotheses about why this difference between studies could occur: 1) the statistical methods used impact the measured treatment effect and estimated ecosystem carbon stocks, and
2) the method used to remove SIC from these soils prior to measuring %C impacted the resulting SOC concentrations.

METHODS

Site Description

The Nevada Desert FACE Facility (NDFF) is located in the northern Mojave Desert in the Great Basin of the Basin and Range physiographic province on the US Department of Energy’s Nevada Test Site in southern Nevada, USA. The arid climate is characterized by limited precipitation (mean annual precipitation=140 mm/yr), low humidity, and large diurnal temperature fluctuations (Jordan et al. 1999; Soper et al. 2017). Soils are Aridisols formed in calcareous alluvial fans resulting in highly variable coarse contents and textures across the site and throughout the profile, but generally ranging from loamy sands in the A horizons (0-0.16 m) to coarse sands with depth (Jordan et al. 1999). There is no caliche layer such that soils are well drained and pH is in the range of 8-9 at all depths (Jordan et al. 1999).

Vegetation of the NDFF is an open, desert scrub formation typical of the Mojave Desert at mid- to low-elevations (Smith et al. 1997). The dominant vegetation at the NDFF is the long-lived evergreen shrub *Larrea tridentata*, followed by the drought-deciduous shrubs *Lycium andersonii*, *Lycium pallidum*, and *Ambrosia dumosa* (Jordan et al. 1999). Collectively, these four species comprise >70% of the shrub population (Jordan et al. 1999). Perennial grasses, primarily *Pleuraphis rigida* also make up an important part of the community. The remaining unvegetated space, known as “interspace” represents greater than 75% of the land cover and is predominately occupied by biological soil crusts (Webb et al. 2003). The Mojave Desert receives low annual precipitation that can be sporadic throughout the winter months such that water is the primary limitation to plant growth (Turner and Randall 1989).
Decommissioning of the NDFF after a decade of exposure to eCO$_2$ provided the opportunity for the destructive sampling of an otherwise intact and undisturbed Mojave Desert ecosystem for the evaluation of the response of SOC to eCO$_2$. The facility was comprised of 9, 23-m diameter circular plots (referred to as “rings” hereafter): three ambient CO$_2$, three elevated CO$_2$ and three control plots to ensure CO$_2$ blowers did not affect results. CO$_2$ treatment began April of 1997 at 550 ppm CO$_2$ and was terminated in June of 2007. Due to unusually low winter precipitation in 2006-2007, all rings were irrigated in March 2007 (30 mm) to stimulate biomass growth before ceasing CO$_2$ treatment. All aboveground biomass and select belowground biomass samples were harvested at the end of the 10-year experiment (Newingham et al. 2013).

Sample Collection

During decommissioning of the NDFF, soil samples were collected following the removal of biological soil crusts and vegetation from the site. Mineral soils were sampled to 1 m in 20 cm increments from areas previously beneath the five dominant forms of vegetation as well as from plant interspaces (Table 1.1). Samples were air-dried and passed through a 2 mm sieve to eliminate coarse fragments. Duplicate soil samples were transported to multiple research labs including ours in Ithaca, NY and stored in watertight containers until analyses. Prior to analysis soils were homogenized and a 20 g sample was in a steel-ball mill. All subsequent analyses were conducted using soil from the homogenized sample.

Soil analysis

Soil samples were pretreated for inorganic C removal using the acid fumigation method described in Jensen & Sparks (in prep). Briefly, soils were weighed at 20 mg directly into silver
capsules on a microbalance (Sartorius Cubis, Germany) and placed in a 96 well plate. Approximately 10 uL of DI water was added to each sample. The well-plate was then placed in a vacuum desiccator along with two open 100 mL beakers of 12 M HCl. The desiccator was sealed with silicon grease to avoid contamination associated with organic sealants (Schubert and Nielsen 2000). The desiccator was placed under vacuum using a hand-held vacuum pump and then closed. Samples were fumigated for 24 hours. After removal from the desiccator, well-plates were dried at 60°C for 24 hours, then double wrapped in tin before dry combustion.

In order to compare this method with SIC removal by acid washing, which has been used previously (Evans et al. 2014; Koyama et al. 2018, 2019), samples from 0-20 cm were treated with the H₃PO₄ method referenced in several other analyses of these soils (Evans et al. 2014; Koyama et al. 2019). We chose to analyze samples from 0-20 cm because this is where SOC concentrations are highest and most likely to show differences by CO₂ treatment. We used three different individuals of each cover type within each ring for an n= 9. This assumes that the level of treatment effect is being tested on the individual plant rather than at the level of the plot.

Pretreatment with H₃PO₄ acid washing was conducted as describe in Jensen & Sparks (in prep) which was based on methods described in previous analysis of NDFF samples (Schaeffer et al. 2003). One g sample of soil was weighed into a glass scintillation vial and treated with three 2 mL aliquots of 1M H₃PO₄. After each addition, soils were shaken and allowed to settle for a minimum of two hours before the acid was removed. The third addition of acid was allowed to sit overnight. After acidification, the same process was repeated with DI water to remove acid that is damaging to the combustion column of the IR-MS. After this rinsing process, soils were dried in an oven at 60°C for 48 hours and then weighed again. The mass loss was used to adjust final %C measurements to the pre-acidification %C concentration. Soils were weighed at 20 mg
into tin capsules for analysis by IR-MS. We analyzed data from each pretreatment method separately to emulate two separate data collections, rather than pooling data for analysis with one model. Mean SOC and δ^{13}C values were analyzed using the same statistical model presented in the “Statistical Analysis of SOC Stocks” section.

All samples, irrespective of acid pretreatment, were analyzed for δ^{13}C and %C using a continuous flow isotope ratio mass spectrometer (IR-MS; Model V Advantage; Thermo Scientific, Bremen, Germany) and elemental analyzer (Thermo Finnigan Carlo Erba NC2500). Isotope ratios are expressed as δ values (per mil):

\[ \delta^{13}C = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000(\text{‰}) \]

where \( R_{\text{sample}} \) and \( R_{\text{standard}} \) are the ratios of heavy to light isotope of the sample relative to the international standard Vienna-Pee-Dee Belemnite. All isotopic analyses were completed at the Cornell University Stable Isotope Laboratory (COIL).

Statistical Analysis of SOC Stocks

We scaled SOC concentrations (mg C•g soil\(^{-1}\)) to stocks (g C•m\(^{-2}\)) using bulk density (kg soil•ha\(^{-1}\)). Bulk density was measured from two pits within each ring. Soil mass was measured at each depth for each ring but was not specific to cover type. SOC concentrations were paired with ring and depth specific bulk density (an average of soil mass from the two pits) to calculate the C stock at each depth. All five depth increments (0-100 cm) were then added together to generate a 1 m SOC stock for each cover type within each ring.

The carbon stock variable (Cstock) was analyzed in a Bayesian framework, implemented with the “Rstan” package, which uses Hamiltonian Markov chain Monte Carlo for model estimation (2020). We labeled each individual stock value \( y_i \) with the identifier \( i = 1, \ldots, n \), where \( n = 173 \) due to 7 missing samples. Associated with each value is an identifier for cover
type \( v(i) = 1, 2, 3, 4, 5, \) or 6\), an identifier for ring \( r(i) = 1, 2, 3, 4, 5, \) or 6\), and an identifier for treatment \( t(i) = 1 \) or 2\). Our model contains an interaction between cover type and treatment,

\[
y_i = a_0 + b_{v(i)} + c_{r(i)} + d_{v(i), t(i)} + e_i
\]

where \( e_1, \ldots, e_n \) are independent normals with mean 0 and variance \( \sigma_1^2 \), and the ring effects are also independent normals with mean zero and variance \( \sigma_2^2 \). We specify half-cauchy priors for the \( \sigma_1^2 \) and \( \sigma_2^2 \), and independent normal priors for \( a_0, b_{v(i)} \), and \( d_{v(i), t(i)} \). Priors were non-informative where \( b_0 \) has a normal prior with mean 0 and standard deviation 10000. Cover type effects and treatment effects have normal priors with mean 0 and standard deviation 1000. The ring effects were modeled as normal with mean 0 and standard deviation parameter \( \sigma_{\text{ring}} \), where \( \sigma_{\text{ring}} \) has a half Cauchy prior with scale 1000. The residuals were modeled with mean zero and standard deviation \( \sigma_{\text{resid}} \), where \( \sigma_{\text{resid}} \) has a half Cauchy prior with scale 1000. For example, under this parameterization, the treatment effect for cover type 3 is \( d_{3, 2} - d_{3, 1} \), meaning that each cover type is allowed to have different treatment effects. We also consider the additive model in which the treatment effects are forced to be equal for each cover type (i.e. the change in SOC under eCO\(_2\) is constrained to be equal for \( L. \) tridentata, interspace, and all other cover types) as is used in the only other published paper assessing C stocks (Evans et al. 2014). We judge statistical significance with 90% credible intervals.

Stocks by cover type were then used to calculate an ecosystem SOC stock by Monte Carlo sampling from the posterior distribution for the treatment effects and scaling them to the ecosystem level using the areal coverage of the three plants with the largest cover: \( L. \) tridentata (12.11%), \( A. \) dumosa (8.92%) and \( L. \) andersonii (1.96%). We were not able to obtain vegetation
cover information from the original NDFF experiment and estimated areal coverage was obtained from nearest USGS long-term vegetation monitoring plot (Beatley plot #15- Jackass Flats) (28). *Pleurapis rigida* and *L. palladium* were not abundant enough to be included in this report and were excluded from ecosystem carbon calculations. The remaining aerial coverage of the ecosystem (100% minus *L. tridentata, A. dumosa, and L. andersonii* coverage) was designated as Interspace (77.01%).

**RESULTS**

*Ecosystem carbon stocks*

Ecosystem SOC stocks as estimated from the set of samples used in the current study show no effect of CO$_2$ treatment using either the interaction or additive models (Fig 2.1). The estimated treatment effect from the interaction model was -191.48 g C•m$^{-2}$ or -16.58% while the additive model estimate was a CO$_2$ treatment effect of -278.06 g C•m$^{-2}$ or -23.50%. For the interaction model, mean total ecosystem organic carbon for the ambient treatment was 1154.81 g C•m$^{-2}$ (90% credible interval: 850.65 – 1440.94 g C•m$^{-2}$) and for the elevated CO$_2$ treatment was 958.79 g C•m$^{-2}$ (90% CI: 613.38 – 1321.12 g C•m$^{-2}$). Using an additive model, the ecosystem carbon stock for the ambient treatment was 1183.71 g C•m$^{-2}$ (90% CI: 896.20 – 1458.01 g C•m$^{-2}$) and the elevated CO$_2$ treatment was 902.69 g C•m$^{-2}$ (90% CI: 611.78 – 1201.59 g C•m$^{-2}$) (Fig 2.1).

*Carbon stocks by cover type*

CO$_2$ treatment effects vary by cover type when the interaction statistical model was used to analyze carbon stocks (Table 2.1; Figure 2.2). The only significant treatment effect was a loss
of -541.45 g C\(\cdot\)m\(^{-2}\) from \textit{L. tridentata} soils (90\% CI: -1044.85 – -37.64 g C\(\cdot\)m\(^{-2}\)). All other cover types show no treatment effects of eCO\(_2\) on carbon stocks. \textit{Ambrosia dumosa} (-343.65 g C\(\cdot\)m\(^{-2}\), 90\% CI: -841.06 – +157.46 g C\(\cdot\)m\(^{-2}\)), \textit{L. andersonii} (-328.22 g C\(\cdot\)m\(^{-2}\), 90\% CI: -823.41 – +171.65 g C\(\cdot\)m\(^{-2}\)), \textit{P. rigida} (-301.87 g C\(\cdot\)m\(^{-2}\), 90\% CI: -807.23 – +220.43 g C\(\cdot\)m\(^{-2}\)) and the unvegetated interspace (-105.71 g C\(\cdot\)m\(^{-2}\), 90\% CI: -646.48 – +456.27 g C\(\cdot\)m\(^{-2}\)). \textit{Lycium palladium} is the only cover type with positive, although non-significant, change in SOC (+149.68 g C\(\cdot\)m\(^{-2}\), 90\% CI: -345.08 – +662.4 g C\(\cdot\)m\(^{-2}\)). When the additive model is used to analyze carbon stocks, all cover types show a decrease of -278 g C\(\cdot\)m\(^{-2}\) which is not a statistically significant treatment effect (Table 2.1; Fig 2.3).
Table 2.1 Elevated CO2 treatment effects as mean (g C•m\(^{-2}\)) and percent change in soil organic carbon stock for six cover types sampled at the Nevada Desert FACE Facility. Treatment effects for the Evans et al study were calculated from the reported mean (g C•m\(^{-2}\) to 1 m depth) while effects reported from the current study are model estimated means.

### Evans et al 2014 | Jensen et al (current study)

<table>
<thead>
<tr>
<th>Cover Type</th>
<th>Evans et al 2014</th>
<th>Jensen et al (current study)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Additive Model</td>
<td>Interaction model</td>
</tr>
<tr>
<td></td>
<td>(Treatment + plant)</td>
<td>(Treatment(^\ast)plant)</td>
</tr>
<tr>
<td>L. tridentata</td>
<td>+222(^\dagger)</td>
<td>-542(^\ast)</td>
</tr>
<tr>
<td></td>
<td>+20%(^\dagger)</td>
<td>-28%(^\ast)</td>
</tr>
<tr>
<td>L. andersonii</td>
<td>+212(^\dagger)</td>
<td>-332</td>
</tr>
<tr>
<td></td>
<td>+20%(^\dagger)</td>
<td>-20%</td>
</tr>
<tr>
<td>L. pallidum</td>
<td>+199(^\dagger)</td>
<td>+148</td>
</tr>
<tr>
<td></td>
<td>+20%(^\dagger)</td>
<td>+8.6%</td>
</tr>
<tr>
<td>A. dumosa</td>
<td>+163(^\dagger)</td>
<td>-348</td>
</tr>
<tr>
<td></td>
<td>+20%(^\dagger)</td>
<td>-28%</td>
</tr>
<tr>
<td>P. rigida</td>
<td>+109(^\dagger)</td>
<td>-311</td>
</tr>
<tr>
<td></td>
<td>+20%(^\dagger)</td>
<td>-25%</td>
</tr>
<tr>
<td>Interspace</td>
<td>+161(^\dagger)</td>
<td>-115</td>
</tr>
<tr>
<td></td>
<td>+20%(^\dagger)</td>
<td>-14%</td>
</tr>
<tr>
<td>Ecosystem</td>
<td>+165(^\dagger)</td>
<td>-191</td>
</tr>
<tr>
<td></td>
<td>+20%(^\dagger)</td>
<td>-17%</td>
</tr>
</tbody>
</table>

\(^\dagger\)Indicates significant difference between control and elevated CO\(_2\) treatments (Bayesian p < 0.05) as reported in Evans et al 2014.

\(^\ast\)Indicates mean CO\(_2\) treatment effect as calculated in the current study does not overlap with zero.

Comparison of SIC Removal Methods

The fumigation method of acid pretreatment results in losses of SOC under eCO\(_2\) for *L. tridentata* (mean = -181.6 g C•m\(^{-2}\), 90% CI: -321.7 – -37.2 g C•m\(^{-2}\)) and *L. andersonii* (mean = -226.5 g C•m\(^{-2}\), 90% CI: -368.0 – -81.5 g C•m\(^{-2}\)) in the top 20 cm of soil. Similarly, using acid washing we found the same two cover types showed significant losses of SOC (*L. tridentata* (mean = -195.9 g C•m\(^{-2}\), 90% CI: -320.1 – -70.32 g C•m\(^{-2}\)) and *L. andersonii* (mean = -134.9 g C•m\(^{-2}\), 90% CI: -256.0 – -10.8 g C•m\(^{-2}\)). No other cover types exhibited significant changes in SOC stocks for either pretreatment method. However, acid washing consistently resulted in lower SOC stocks for both control and eCO\(_2\) soils, as has been shown in previous analyses of
these SIC removal methods (Jensen & Sparks, *in prep*). Elevated CO$_2$ resulted in a decrease in $\delta^{13}$C for *L. pallidum* when pretreated with acid washing (mean = -0.86‰, 90% CI -1.58‰ – -0.17‰). When soils were pretreated with fumigation, *L. andersonii* (mean = -2.66‰, 90% CI -4.16‰ – -1.02‰) and *P. rigida* (mean = -3.54‰, 90% CI -5.09‰ – -1.87‰) exhibited a decrease in $\delta^{13}$C.

**DISCUSSION**

In this analysis, ten years of elevated atmospheric CO$_2$ in the Mojave Desert resulted in a decrease in the SOC stock for the major plant cover type in the ecosystem, *L. tridentata*. The loss of 541.45 g C•m$^{-2}$, when scaled by the areal coverage of *L. tridentata* (12.1%) and the area of the Mojave Desert, equates to a loss of ~8.1 Megatons of SOC. Given that *L. tridentata* is the most abundant perennial shrub in the warm deserts of North America, its influence on carbon cycling may be large in a global context (Jordan et al. 1999). Further, this estimate may be conservative as the area associated with *L. tridentata* is assumed to stop at the edge of the canopy while the rooting system of these shrubs are known to extend up to 2 m away from the shrub stem into the interspace (Brisson and Reynolds 1994). Understanding the relationship between the aboveground size of the shrub canopy and the belowground extent of the island of fertility effect would provide more accurate measurements of carbon stocks in this ecosystem.

One potential explanation for the observed decrease in SOC for *L. tridentata* soils is a priming mechanism, where an increase in carbon to the soil ecosystem results in microbial decomposition greater than the amount of new carbon inputs, resulting in a net loss of SOC (Kuzyakov et al. 2000). Drought deciduous shrubs at the NDFF increased photosynthesis in monsoon years during the experiment, providing an increase in carbon to the ecosystem, without long term changes in biomass (Newingham et al. 2013). Priming may then occur when this new
photosynthate, as rhizosphere carbon flux or litter leachate, is used to mineralize existing organic matter in order to release other nutrients such as nitrogen (Finzi et al. 2006; Carney et al. 2007; Phillips et al. 2011). This effect may be particularly prominent in ecosystems characterized by low soil clay content and recalcitrant litter (Sulman et al. 2014). A shift in microbial carbon use under eCO$_2$ potentially has long-term consequences for organic carbon mineralization and stabilization in this ecosystem (Crow et al. 2009; Sollins et al. 2009; Manzoni et al. 2018).

No other cover types showed differences in SOC stocks between control and elevated CO$_2$ treatment plots, and no overall change in ecosystem SOC stock. This result contrasts with previously published results from the NDFF and highlights the importance of statistical and methodological decisions for studies in this ecosystem. This study provides a unique situation of having replicated measurements using different methods, allowing us to explore the impact of these decisions through direct comparison. Here, we put forward two potential causes for the differences between the present study and previously published data and attempt to assess the roles they play in the discrepancy. The first is an assessment of the statistical analysis and the second is a discussion of the impacts of SIC removal methods.

Comparison of Statistical Analyses

Arid ecosystems are spatially variable and well-established studies of desert ecology have shown that distinct biogeochemical cycling occurs in soils below perennial shrub canopies and unvegetated soils (Schlesinger and Pilmanis 1998). Thus, soils associated with plant cover likely show different responses to eCO$_2$ compared to unvegetated soils and accurately assessing the effect of eCO$_2$ on ecosystem SOC requires accounting for those different responses. Soils from NDFF allows us to do that as samples were taken from distinct cover types at the site. The model
underlying the analysis in Evans et al 2014 does not allow for this variation among cover types despite the capacity to incorporate this information in ecosystem SOC stock estimates. The model used in Evans et al 2014 results in the same relative CO$_2$ treatment effect for all cover types, including interspace, as seen with the consistent 20% increase in SOC for all cover types. (Table 2.2). Our alternative statistical model includes an interaction term between ‘treatment’ and ‘cover type’, which allows us to test for different responses among cover types (i.e., an interaction model) (Table 2.2). The results of the interaction model show that treatment effects do indeed vary substantially among cover types, justifying the need for that interaction term.

To understand the impact of the statistical choice in Evans et al (2014) we analyzed our data with an additive model as well. Our additive model results a treatment effect in terms of g C•m$^{-2}$ that is the same across cover types where the Evans model results in a consistent treatment effect in terms of a percent change (Table 2.1). With our model, we found no significant treatment effect on SOC stocks for any cover type (mean treatment effect = -278 g C•m$^{-2}$; 90% CI -603.6 – +57.65) (Fig 2.3). Using this model, the CO$_2$ treatment effect for most of the vegetative cover types is underestimated. In the case of L. tridentata, the change in SOC estimated from the additive model is not a statistically significant loss which contrasts with the results of the interaction model. Lycium pallidum is one example of where the choice of statistical model results in opposite treatment effects: an increase of +148 g C•m$^{-2}$ with the interaction model and a decrease of 278 g C•m$^{-2}$ with the additive model, although neither of these changes are statistically different from zero. Finally, the decrease in SOC for interspace soils under eCO$_2$ is about 60% greater in the additive model relative to the interaction model (Table 2.2). Given that interspace covers approximately 80% of this region of the Mojave Desert, this overestimate is likely the most consequential impact of the statistical model choice.
The ecosystem SOC stock treatment effect produced from the additive model is $-278$ g C•m$^{-2}$ as a result of all cover types having the same treatment effect (Fig 2.1; Eq 2.2). The difference from the interaction model ecosystem treatment effect ($-191$ g C•m$^{-2}$) is due primarily to the weight of the interspace treatment effect in calculating the ecosystem treatment effect. The fundamental calculation behind the ecosystem carbon stocks can be understood with a basic weighted means equation, where the treatment effect for each cover type is weighted by the proportion of land area (Eq 2.1). When all cover types have the same treatment effect, as with the additive model (Fig 2.2), the treatment effect for the ecosystem is the same regardless of the amount of area each plant type covers (Eq 2.2). When treatment effects differ among cover types (Fig 2.3), the ecosystem carbon stock appropriately reflects the proportion of a given area.

Fig 2.1 Mean ecosystem C stocks (g C•m$^{-2}$ to 1 m depth) using an interactive statistical model and an additive statistical model. Elevated CO$_2$ treatment effect in the interactive model is $-191$ g C•m$^{-2}$ (90% CI = $-638$ g C•m$^{-2}$ – $+260$ g C•m$^{-2}$) and the additive model is $-278$ g C•m$^{-2}$ (90% CI= $-603.6$ – $+57.65$). Thick vertical bar is 50% confidence interval, thin vertical bar is 90% confidence interval.
affected by each (Eq 2.3). The treatment effect of eCO$_2$ at the ecosystem level should be much closer to the effect on interspace because it represents 77% of the ecosystem.

**Eq 2.1** Ecosystem treatment effect weighted by cover type spatial coverage

\[
\text{Treatment Effect} = .77(\Delta SOC_{\text{INTSP}}) + .12(\Delta SOC_{\text{LATR}}) + .09(\Delta SOC_{\text{AMDU}}) + .02(\Delta SOC_{\text{LYAN}})
\]

**Eq 2.2** Additive Model

\[
.77(-278) + .12(-278) + .09(-278) + .02(-278) = -278 \text{ g C} \cdot \text{m}^{-2}
\]

**Eq 2.3** Interaction Model

\[
.77(-115) + .12(-542) + .09(-348) + .02(-332) = -191 \text{ g C} \cdot \text{m}^{-2}
\]

Fig 2.2 Mean soil organic carbon stock (g C•m$^{-2}$ to 1 m depth) from an additive statistical model. Thick vertical bar is 50% confidence interval, thin vertical bar is 90% confidence interval. All cover types have the same, non-significant CO$_2$ treatment effect of -278 g C•m$^{-2}$ (90% CI -603.6 – +57.65).

An additive model erases important details that inform our understanding of how eCO$_2$ affects SOC stocks in this ecosystem. The incorporation of unique treatment effects for each cover type results in a decrease from -24% ecosystem SOC in the additive model to -17% with
interactive model (Table 2.2). We believe the interactive model is more appropriate for understanding CO$_2$ treatment effects in this ecosystem, however, the difference in models used does not explain why the previous analysis showed an increase in SOC while our results show no change in ecosystem SOC stock.

\[ \text{Fig 2.3 Mean soil organic carbon stock (g C•m}^{-2}\text{) by cover type using an interaction model. Thick vertical bar is 50\% confidence interval, thin vertical bar is 90\% confidence interval. Asterisk above L. tridentata indicates the estimated CO}_2\text{ treatment effect does not overlap with 0.} \]

\textit{Carbonate Removal Methods}

The effect of eCO$_2$ on SOC stocks did not vary between the SIC pretreatment methods of acid washing and fumigation (Fig 2.5). One of the only methodological differences between the two studies being compared here is the method used for removal of SIC before SOC analysis. Acid washing with phosphoric acid ($\text{H}_3\text{PO}_4$) has been shown to result in a loss of up to 36\% of organic carbon relative to the fumigation method in these soils (Jensen & Sparks, \textit{in prep}). The key difference between the two pretreatment methods is that direct addition of $\text{H}_3\text{PO}_4$ to soil involves removal of liquid at multiple points in the process, meaning that this method has a
pathway for carbon loss while fumigation with HCl does not. Soil organic C may be lost during acid washing through two major pathways. First, particulate organic matter that is less dense than the liquid used in acid washing will remain suspended during acidification and can be decanted along with the acid solution or water used to rinse the soil. Second, carbonates can stabilize SOC through divalent (Ca\(^{2+}\)) chemical bonds with organic matter or through secondary carbonate coatings (Baldock and Skjemstad 2000), which may be disrupted with acidification (Fernandes and Krull 2008). This loss of SOC during acid washing may provide an explanation for why SOC stocks measured in this study are larger than in Evans et al 2014.

Direct comparison of SOC stocks from data in the current study to previously documented SOC stocks shows that the difference in SOC stocks is most clear in the samples from the ambient CO\(_2\) treatment samples (Fig 2.4). Averaged across all cover types, we measure \(~500 \) g C\(\cdot m^{-2}\) or 33% more SOC in soils from control CO\(_2\) plots, but measured approximately the same amount of carbon in soils from the eCO\(_2\) treatment. The lower SOC stock in the control measured by Evans et al (2014) could create an apparent increase in SOC when compared to eCO\(_2\) soils. One hypothesis that could explain this difference is an asymmetric loss of SOC during SIC removal where more SOC is lost from soils from control CO\(_2\) plots than elevated CO\(_2\) plots. This could happen if eCO\(_2\) affects the amount of light particulate matter or carbonate stabilized SOC in these soils.
To test this hypothesis, we treated soils with both methods (acid washing and fumigation) and report SOC (g C•m\(^{-2}\)) to 20 cm depth resulting from each. We chose to only test samples from 0-20 cm in the soil profile because that is where the SOC concentrations are highest and SIC removal may have the largest impact on the SOC pool. The effect of elevated CO\(_2\) on SOC stocks did not vary across acid methods (Fig 2.5) but acid washing consistently results in lower SOC stocks for both control and eCO\(_2\) soils, which is consistent with previous analyses of these SIC removal methods (Jensen & Sparks, in prep). One potential explanation for the higher SOC stocks in fumigated soils could be that this method does not fully remove SIC, resulting in higher %C after acidification. However, stable isotope analysis of these soils shows that fumigation results in a more depleted \(\delta^{13}C\) compared to acid washing (Fig 2.6). Soil inorganic carbon in

---

**Fig 2.4** Soil organic carbon stocks (g C•m\(^{-2}\) to 1 m depth) from the current study and the Evans et al 2014 study. Boxplot was created from the mean SOC stock for each cover type (n=6) presented in the Evans et al 2014 supplementary information (white boxes) and from the current study (grey boxes). Raw data were not used because it is not available for the Evans et al study. This figure highlights the greater difference between studies in the ambient CO\(_2\) (control) treatment SOC stocks relative to the elevated CO\(_2\) (+CO\(_2\)) treatment SOC stocks.
these soils is enriched (+1 to -2 ‰) relative to SOC (Rovira et al. 2019). If fumigation failed to remove SIC we would expect to see a less negative δ¹³C. These results indicate that the SOC concentrations and δ¹³C values reported in Evans et al 2014 may be different due to the loss of SOC during the acid washing process, and that the SOC stocks reported in the 2014 paper are likely underestimates. However, this again does not explain the opposite treatment effects measured in each study.

Fig 2.5 Mean soil organic carbon stocks (g C•m⁻²) to 20 cm depth resulting from two methods of soil inorganic carbon removal, acid washing with H₃PO₄ and fumigation with HCl. Elevated CO₂ treatment effects do not vary by SIC removal method, but fumigation estimates show SOC stocks 20-30% greater than acid washing. Thick vertical bar is 50% confidence interval, thin vertical bar is 90% confidence interval. Asterisks indicate that estimated CO₂ treatment effects do not overlap with 0.
CONCLUSION

Results from our alternative analysis of soils from the NDFF show that the eCO₂ treatment effect on ecosystem SOC stocks was a loss of 191.48 g C·m⁻² or -16.58%. Although this loss was not significant, it provides a stark contrast to the +165 g C·m⁻² or +20.50% change in ecosystem SOC reported in Evans et al (2014). We attempted to understand the difference between studies by assessing the statistical analysis of each data set and the methods used for measurements of SOC. Unfortunately, neither of these fully explain the discrepancies. One potential explanation is that the variation in SOC among individual plants is extremely high at NDFF. Although the NDFF was designed for one soil sample per ring per cover type, during the final destructive harvest at the site soils were collected from all plants in each ring, resulting in a sample set containing multiple soils from the same cover type. Therefore, it is possible that across analyses different individual soil samples were selected and the variation in SOC
concentrations among these samples explains some amount of discrepancy among studies. However, it seems unlikely this difference would be large enough to explain the differences between this study and Evan et al 2014. Another possibility is that samples were mislabeled, or plots treatments misinterpreted. However, in that scenario we would expect a completely opposite data set, rather than the asymmetric differences across CO₂ treatment highlighted in Figure 4. Regardless of the cause, this comparison highlights the imperative need to publish full data sets and analysis code so that inconsistencies among studies can be rectified quickly.

Since Evans et al. 2014 was published, other studies have attempted to explore the mechanisms behind the increase in SOC; the interpretation of other analyses of these soils, such as microbial enzymes or organic matter composition, are influenced by the assumption that these desert soils take up carbon under eCO₂ (Evans et al. 2014; Koyama et al. 2018, 2019). The NDFF is also cited in several meta-analyses that attempt to describe large patterns across many ecosystem studies and could be creating misleading trends (Terrer et al. 2016, 2018, 2019). Given the importance of arid ecosystems in global carbon cycling and the limited number of eCO₂ studies conducted in deserts, the conflict in these data from the NDFF must be rectified to accurately predict global soil carbon under future climate scenarios.
REFERENCES


CHAPTER THREE

ELEVATED ATMOSPHERIC CO₂ DRIVES CHANGES IN SOIL ORGANIC MATTER POOLS IN A DESERT ECOSYSTEM: EVIDENCE FROM A PHYSICAL FRACTIONATION AND ORGANIC COMPOUND ANALYSIS
ABSTRACT

Quantifying changes in pools of soil organic carbon (C) under disturbance is critical to modeling future global change. In this study, we conducted a novel soil fractionation procedure on soils previously harvested from the Nevada Desert FACE Facility in the Mojave Desert to further our understanding of soil C stability under elevated carbon dioxide (CO$_2$). Soils were subjected to a sodium polytungstate density fractionation (1.85 g/cm$^3$) to separate light, particulate organic matter (POM, < 1.85 g/cm$^3$) from heavier, mineral associated organic matter (MAOM, > 1.85 g/cm$^3$). These two fractions were analyzed for organic C and concentration, as well as $\delta^{13}$C. Further separation of a < 20 µm fraction was analyzed by pyrolysis GC/MS to qualitatively assess changes in chemical composition of MAOM.

Our results show that when exposed to elevated CO$_2$ for 10 years soils showed a significant overall decrease of C in POM for the dominant cover type in this ecosystem, *Larrea tridentata*. This trend is significant in soils from 20-40 cm in depth and is also seen in stocks up to 1 m in depth (g • m$^{-2}$). MAOM-C stock did not vary with CO$_2$ treatment, but pyrolysis GC/MS analysis indicated a slight increase in lipid compounds associated with microbial biomass, indicating a possible change in microbial carbon use. Both POM-C and MAOM-C were depleted in $\delta^{13}$C in the elevated CO$_2$ treatment, indicating incorporation of the more depleted $\delta^{13}$C-CO$_2$ source for CO$_2$ fumigation (-44‰). Given that previous studies at the Mojave FACE measured increased litter inputs, our results indicate accelerated decomposition of POM under elevated CO$_2$. Further experiments are needed to understand the long-term implications of a decrease in fast-cycling POM under global change and how this may impact slow-cycling MAOM on a longer time scale.
INTRODUCTION

Anthropogenic climate change has accelerated the movement of carbon (C) between geological and biological pools disturbing multiple aspects of the global C cycle. Elevated atmospheric CO$_2$ impacts the terrestrial C cycling by increasing rates of photosynthesis. This consequently affects the flux of organic C into an ecosystem and can influence aboveground biomass, belowground biomass, and soil organic carbon (SOC) pools. Soil organic C is the largest stock of terrestrial organic C, storing more than 1500 Pg of C in the top 1 m, accumulated from the decomposition of organic matter (Jobbágy and Jackson 2000; Friedlingstein et al. 2020). The quantity of C stored as soil organic matter is related to atmospheric CO$_2$ concentrations by the movement of CO$_2$ to the biosphere as Net Primary Productivity (NPP) and back to the atmosphere via heterotrophic respiration. These two processes dictate the size of the SOC pool and are fundamental to modeling the global C cycle. Disturbances such as climate change alter the balance of these fluxes but there is high uncertainty in the extent to which this impacts SOC cycling and stocks (Todd-Brown et al. 2013, 2014; Bradford et al. 2016).

Long-term studies of elevated CO$_2$ (eCO$_2$) have shown that SOC can increase (Jastrow et al. 2005), decrease (Carney et al. 2007), or to not respond to this disturbance (Ellsworth et al. 2017; Carrillo et al. 2018). However, less is known about how these changes in stocks are related to changes in SOC stability. Stability reflects the turn-over time of C, or how quickly C cycles through the soil environment and back into the atmosphere via heterotrophic respiration. Elevated CO$_2$ indirectly can affect SOC stability by causing a shift in C allocation among C sources such as leaves, roots, or rhizodeposition (Drake et al. 2011; Sulman et al. 2014; Terrer et al. 2021). Microbial mineralization and use of SOC is dependent on the type of organic matter input (structural versus simple organic compounds), among other factors (Schimel and Schaeffer 2012). Turn-over time of SOC is therefore linked to atmospheric CO$_2$ concentrations, making the
stability of SOC stocks a critical component of the global C cycle.

To understand eCO$_2$ impacts on C stability it is useful to consider SOC in two pools of contrasting stability (Lavallee et al. 2020) (Table 3.1). Particulate organic matter (POM) is derived from larger plant detritus that has undergone minimal decomposition and is considered less stable (i.e. faster turn-over). This C is more susceptible to microbial mineralization and may be rapidly respired back into the atmosphere (Sollins et al. 2006; Throop et al. 2013; Gianello and Bremner 1986). In contrast, mineral associated organic matter (MAOM) is made up of highly decomposed and microbially processed small organic compounds bonded to mineral surfaces through strong chemical associations (Kallenbach et al. 2015). Carbon in this form is more stable against mineralization than POM and will have, on average, a longer residence time in the soil (Sokol et al. 2019). POM is formed directly from litter inputs while MAOM is formed directly from dissolved organic carbon or indirectly through microbial decomposition of POM (Sokol and Bradford 2019). Because POM and MAOM are formed from different sources of organic matter and have distinct turn-over times, changes in these pools can reveal how C allocation impacts SOC stability.

Table 3.1 Characteristics of particulate and mineral associated organic matter relevant function of organic matter pool and physical separation. Table adapted from Lavallee et al 2019.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Particulate Organic Matter</th>
<th>Mineral Associated Organic Matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
<td>Fragmented plant material</td>
<td>Dissolved organic carbon, microbial byproducts</td>
</tr>
<tr>
<td>Density</td>
<td>Light (&lt; 1.6 - 1.85 g•cm$^{-3}$)</td>
<td>Heavy (&gt; 1.6 - 1.85 g•cm$^{-3}$)</td>
</tr>
<tr>
<td>Size</td>
<td>Larger organic material, not water extractable (&gt; 20-63 µm)</td>
<td>Molecules bound to mineral surfaces (&lt; 20-63 µm)</td>
</tr>
<tr>
<td>C:N</td>
<td>Higher C:N</td>
<td>Lower C:N</td>
</tr>
<tr>
<td>$\delta^{13}$C</td>
<td>Depleted (more negative)</td>
<td>Enriched (less negative)</td>
</tr>
</tbody>
</table>
Loss of SOC under eCO$_2$ is often attributed to a priming mechanism where the introduction of low molecular weight C compounds (as litter leachate or root exudates) stimulate microbial activity and the decomposition of pre-existing soil organic matter (Kuzyakov et al. 2000). When the decomposition of old SOC outweighs the input of new C there is a net loss of SOC. In nitrogen (N) limited ecosystems, priming can be a result of microbial demand for N. MAOM definitionally has a higher concentration of N and so can be the target of microbial priming, resulting in an increased in C:N of this pool. Quantifying the concentration of N in POM and MAOM, and changes in C:N may reveal if microbial mining of N is the cause of a loss of SOC. Priming may be a particularly prevalent response to eCO$_2$ in soils with low clay content, such as those found in the Mojave Desert, which limits the amount of mineral surface area available for C stabilization and increases the likelihood of microbial uptake (Sulman et al. 2014).

The Mojave Desert Free Air CO$_2$ Enrichment experiment (Mojave FACE) was a long-term elevated CO$_2$ experiment located in an undisturbed desert ecosystem. Ten years of eCO$_2$ resulted in increased leaf-level photosynthesis (Naumburg et al. 2003), did not have any sustained effects on plant biomass (Newingham et al. 2013), but did result in a loss of SOC from soils under the dominant shrub, *Larrea tridentata* (Jensen & Sparks, unpublished). Most of the Mojave Desert is not vegetated and this “interspace” soil exhibited no change in SOC stock at the FACE site. The contrasting SOC responses of these two cover types to eCO$_2$ at Mojave FACE present an opportunity to explore eCO$_2$ mediated changes to SOC stocks and stability by assessing changes in POM and MAOM. The unique vegetation distribution of this ecosystem may further allow us to explore the role of above and belowground C inputs by comparing changes in each C pool between vegetated (greater litter inputs and rhizosphere C flux) and
unvegetated soils (little litter input and less rhizosphere C flux).

Here we conduct a two-pool density fractionation of soils from the Mojave FACE experiment to explore changes in POM and MAOM as a means of eCO₂ understanding impacts on SOC stability. We analyze samples from the two dominant vegetation cover types, *L. tridentata* and interspace soils, and at two depths, 0-20 cm and 20-40 cm. Focusing on these two depths allows us to separate out changes driven by aboveground litter inputs relative to belowground rhizosphere C flux. Surface soils are more directly impacted by litter at the surface which affects POM pools directly through physical incorporation of detritus and MAOM indirectly through litter leachate. The deeper soils may more directly indicate rhizosphere effects as *L. tridentata* has secondary lateral roots concentrated at 20-35 cm in the profile and can extend up to 3 m into interspace soils (Singh 1967; Fonteyn and Mahall 1981). Previous studies from the Mojave FACE site showed no changes in root growth under eCO₂ (Evans et al. 2014). Therefore, in soils at the rooting depth (20-40 cm), we would expect most changes to be driven by root exudation of simple C inputs that affect MAOM formation and persistence.

To further understand changes in C cycling under eCO₂ we measure δ¹³C of POM and MAOM pools. At the Mojave FACE site, the CO₂ source used to treat the eCO₂ plots was from a fossil fuel derived source with a δ¹³C of -44‰ compared to the naturally occurring -8‰ atmospheric CO₂. This allows us to trace the new photosynthate from plants exposed to eCO₂ into the soil organic matter pool. New SOC in eCO₂ plots should appear more depleted than C fixed from control plots. Previous analysis of vegetative and root tissue at Desert FACE showed δ¹³C to be depleted for *L. tridentata* in eCO₂ plots (Evans 2014). Although we do not have direct measurements of the δ¹³C of root exudates or leachate at this site, we can assume any new photosynthesize in eCO₂ plots would also be δ¹³C depleted. The δ¹³C data can indicate if new C is
primarily entering POM or MAOM under eCO$_2$. However, we cannot use these data to compare cycling rates with control plots as there was no equivalent $\delta^{13}$C tracer in the CO$_2$ of control plots.

Finally, we analyze MAOM by pyrolysis GC/MS to quantify changes in organic compound composition. We separate a $< 20 \mu$m fraction of MAOM to remove sand size particles and qualitatively assess changes different diagnostic classes of organic compounds (Table 3.2). This method indicates shifts in compounds derived from microbial or plant products and allows us to further understand changes in this most stable pool of organic matter.

Table 3.2 Pyrolysis GC/MS compound class identification. Every identified compound is assigned to a class of origin. Each class of compounds is assumed to be derived from plant or microbial origin or can be found in both. Compounds with and without significant N content are indicated to demonstrate which classes could be depleted under N-mining conditions.

<table>
<thead>
<tr>
<th>Compound Class</th>
<th>Source</th>
<th>N Containing</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aromatic</td>
<td>Both</td>
<td>No</td>
<td>Guggenberger et al. 1995</td>
</tr>
<tr>
<td>Lignin</td>
<td>Plant</td>
<td>No</td>
<td>Grandy et al. 2007</td>
</tr>
<tr>
<td>Lipid</td>
<td>Microbial</td>
<td>No</td>
<td>Grandy et al. 2007</td>
</tr>
<tr>
<td>N-Bearing</td>
<td>Microbial</td>
<td>Yes</td>
<td>Kallenbach et al. 2016</td>
</tr>
<tr>
<td>Phenol</td>
<td>Plant</td>
<td>No</td>
<td>Grandy et al. 2007</td>
</tr>
<tr>
<td>Polysaccharide</td>
<td>Both</td>
<td>No</td>
<td>Martin et al. 1980; Grandy et al. 2007</td>
</tr>
<tr>
<td>Protein</td>
<td>Microbial</td>
<td>Yes</td>
<td>Grandy et al. 2007</td>
</tr>
</tbody>
</table>

We hypothesize that 1) eCO$_2$ will cause a decrease in SOC stability of $L. tridentata$ SOC due to an increase in POM and a loss of MAOM. This may be accompanied by a loss of MAOM-C due to litter-derived DOC stimulating microbial decomposition of SOC via priming (Kuzyakov et al. 2000; Phillips et al. 2011). In contrast, interspace soils will show no response of POM to eCO$_2$ as there is low amounts of litter that remain on unvegetated soils long enough to
be incorporated into SOC (Weatherly et al. 2003). 2) Decreases in MAOM will be concentrated at the 20-40 cm depth and have a larger effect in *L. tridentata* soils than interspace soils. 3) That isotope data will support more rapid cycling of POM as indicated by greater depletion of $\delta^{13}$C in this pool under eCO$_2$ relative to MAOM. And 4) Pyrolysis GC/MS will show microbial mining results in a loss of N-enriched compounds from the < 20 µm fraction of MAOM.

METHODS

*Site Description*

The Nevada Desert FACE Facility (NDFF) is located in the northern Mojave Desert in the Great Basin of the Basin and Range physiographic province on the US Department of Energy’s Nevada Test Site in southern Nevada, USA. The arid climate is characterized by limited precipitation (mean annual precipitation=140 mm/yr), low humidity, and large diurnal temperature fluctuations (Jordan et al. 1999; Soper et al. 2017). Soils are Aridisols formed in calcareous alluvial fans resulting in highly variable coarse contents and textures across the site and throughout the profile, but generally ranging from loamy sands in the A horizons (0-1.6 m) to coarse sands with depth (Jordan et al. 1999). There is no caliche layer such that soils are well drained and pH is in the range of 8-9 at all depths (Jordan et al. 1999).

Vegetation of the NDFF is an open, desert scrub formation typical of the Mojave Desert at mid- to low-elevations (Smith et al. 1997). The dominant vegetation at the NDFF is the long-lived evergreen shrub *Larrea tridentata*, followed by the drought-deciduous shrubs *Lycium andersonii, Lycium pallidum,* and *Ambrosia dumosa* (Jordan et al. 1999). Collectively, these four species comprise >70% of the shrub population (Jordan et al. 1999). Perennial grasses, primarily *Pleuraphis rigida* (PLRI) also make up an important part of the community. Plant interspaces (INSP) represent ~80% of the land cover and are predominately occupied by
biological soil crusts (Webb et al. 2003). The Mojave Desert receives low annual precipitation that can be sporadic throughout the winter months such that water is the primary limitation to plant growth (Turner and Randall 1989).

Decommissioning of the NDFF after a decade of exposure to FACE provided the opportunity for the destructive sampling of an otherwise intact and undisturbed Mojave Desert ecosystem for the evaluation of the response of SOM to eCO$_2$. The facility was comprised of 9, 23-m diameter circular plots (referred to as “rings” throughout): three ambient CO$_2$, three elevated CO$_2$ and three control plots to ensure CO$_2$ blowers did not affect results. Treatment was started in April of 1997 at 550 ppm CO$_2$ and was terminated in June of 2007. Due to unusually low winter precipitation in 2006-2007, all rings were irrigated in March 2007 (30 mm) to stimulate biomass growth before ceasing CO$_2$ treatment. All aboveground biomass and select belowground biomass samples were harvested at the end of the 10-year experiment (Newingham et al. 2013).

Sample Collection

During decommissioning of the NDFF, soil samples were collected following the removal of biological soil crusts and vegetation from the site. Mineral soils were sampled to 40 cm in 20 cm increments from areas previously beneath the canopy of *L. tridentata* plants as well as from the unvegetated interspace. Samples were air-dried and passed through a 2 mm sieve to eliminate coarse fragments. Soils were transported to Ithaca, NY and stored in watertight containers until analyses. Interspace samples from ring 2 (elevated CO$_2$ plot) could not be located at the time of this experiment and so are missing from this data set.

Density Fractionation

The physical characteristics of POM and MAOM allow for separation of these two pools
based on size and density; POM is larger and lighter while MAOM is smaller and denser. Here, we conduct a simple two-pool density fractionation to isolate these two fractions. We conducted this process on soils from L. tridentata and interspace cover types to assess the influence of vegetation on SOC stability. We further sampled soils from two depths of the profile, surface (0-20 cm) versus rooting depth (20-40 cm) of L. tridentata, to understand the effects of aboveground versus belowground C allocation. Previous studies of rooting geometry have shown L. tridentata to have an extensive lateral rooting system around 30 cm that can extend up to 2 m from the base of the shrub (Mudrak; Brisson and Reynolds).

A two-pool sodium polytungstate ([SPT], Low C & N product, Sometu, Berlin, Germany) density fractionation was conducted with the intent of separating free organic matter from the mineral associated organic fraction. A density of 1.85 g/cm³ was chosen based on Throop et al.’s results from a seven-step density fractionation of shrub and interspace soils in the Chihuahua Desert (Sollins et al. 2006b; Throop et al. 2013; Kramer et al. 2017). No dispersion step was used based on other studies showing negligible occluded material in arid ecosystems, attributable to the sandy, quartz mineralogy which inhibits aggregate formation (e.g., (Rasmussen and White 2010). A fresh sample of SPT-0 powder was analyzed by IRMS to ensure C and N levels were below detection limits (Kramer et al 2009).

For each soil sample, 25 g was shaken for 2-3 hours in 30 mL of 1.85 g/cm³ solution of SPT in a 50 mL conical bottom falcon tubes. Samples were centrifuged at 3000 rpm for 30 minutes to attain sufficient separation of floating organic matter. Distinct layers were formed such that all light material floated at the surface, heavier material pelleted at the bottom, and no visible particulate matter floated in between. The light fraction was decanted onto glass fiber filters (PALL Life Sciences, A/E, 1.0 μm), using care to not disturb the surface of the heavy
fraction, and rinsed with at least 100 mL of diH$_2$O. Any remaining POM visible on the surface of the heavy fraction was then removed by hand and added to the light fraction filter. The heavy fraction was also filtered on glass fiber filters and rinsed with at least 250 mL diH$_2$O before drying. All samples were dried for 48 hours at 60°C.

**Size Fractionation**

Size fractionation was carried out on additional sub-samples of soil. Using the same density procedure, the light fraction (< 1.85 g/cm$^3$) was removed from soil prior to size fractionation. The remaining heavy fractions were passed through a 20 µm sieve to separate the sand fraction from clay/silt. Approximately 300 mL of diH$_2$O was used to rinse soils through the filter, plus another 100 mL to transfer to centrifuge buckets. The < 20 µm fraction was centrifuged in 500 mL buckets (Nalgene) at 1000 RPM for 2 hours. The top 200 mL of each sample was decanted, without disturbing soil surface. An additional 200 mL of diH$_2$O was added and samples were centrifuged at 1000 rpm again for 2 hours. Supernatant was decanted and the remaining soil transferred into tins to dry at 60°C for 48 hours.

**Sample Analysis**

Light soil fractions were removed from the filter and ground by hand in a mortar and pestle prior to analysis. A 0.15 g subsample of the 20 µm soil fractions were treated for carbonate removal before analyzing for SOC and total N. Heavy soil fractions were removed from filters and homogenized. Heavy fraction soils were weighed into silver capsules at 20 mg and acidified to remove inorganic carbon using the fumigation method described in Jensen & Sparks, 2022. Briefly, 20 mg of soil were weighed into silver capsules in a 96-well plate and 10 µL of DI H2O added to each sample. The well plate was then placed in a desiccator with a
beaker of 12M HCl for 24 hours under vacuum.

All samples were analyzed for δ\textsuperscript{13}C, %C, δ\textsuperscript{15}N, %N using a continuous flow isotope ratio mass spectrometer (IR-MS; Model V Advantage; Thermo Scientific, Bremen, Germany) and elemental analyzer (Thermo Finnigan Carlo Erba NC2500). Isotope ratios are expressed as δ values (per mil):

\[ \delta^{13}C = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 (\text{‰}) \]

where \( R_{\text{sample}} \) and \( R_{\text{standard}} \) are the ratios of heavy to light isotope of the sample relative to the international standards for C and N, Vienna-Pee-Dee Belemnite and atmospheric N\textsubscript{2}, respectively. All isotopic analyses were completed at the Cornell University Stable Isotope Laboratory (COIL). Soil %C and %N values were scaled to stocks (g*m\textsuperscript{-2}) using bulk density measurements at each depth, averaged across all rings.

Pyrolysis-GC/MS analysis of < 20 μm samples was conducted at the University of New Hampshire. Samples were not pre-treated for carbonates in order to avoid damaging organic compound structures. Compound peaks were analyzed and identified with the Automated Mass Spectral Deconvolution and Identification Systems (AMDIS V 2.65) software, the National Institute of Standards and Technology (NIST) compound library and published literature (Grandy et al., 2009). Organic matter compounds are expressed as the % relative abundance of total sample peak area and classified based on origin (lipids, lignin derivatives, polysaccharides, proteins, non-protein N-bearing and phenolics). Identifiable compounds with potentially multiple origins were classified as unspecified. Aromatic compounds also with an unspecified origin were classified as aromatics (Grandy et al. 2009).

**Statistical Analysis**

All statistical analyses were conducted using the statistics package R, version 3.5.1
Light fraction and heavy fraction data were analyzed separately. We used linear mixed-model analysis of variance (ANOVA) where ‘CO₂ Treatment’, ‘Species’ and ‘Depth’ were fixed effects, and ‘Plot’ was a random effect. We conducted pairwise comparisons between treatments (ambient and elevated CO₂) and between cover types (L. tridentata and interspace) using the pair comparison function in package ‘emmeans’. Residuals were inspected to assess normality and where data did not meet assumptions of normality required for parametric tests, values were log transformed. Analysis of variance was used to assess fixed effects using R package “lme4” (Bates et al. 2015). Post-hoc pairwise comparisons were conducted using the R package “emmeans” (Lenth 2020). Significance was set at alpha = 0.05.

RESULTS

Characterizing Isolated Fractions

Density fractionation of soils from NDFF separated out two distinct pools of organic matter. POM (< 1.85 g•cm⁻³) was a very small fraction of the sample by weight, on average less than 0.1 g out of a 25 g sample while the majority of the sample separated into MAOM (> 1.85 g•cm⁻³), more than 24.8 g on average. POM samples were 17% C on average while MAOM was less than 0.15% C on average. This pattern is likely attributable to the texture of these soils which are approximately 85% sand. Sand retains very little organic matter due to low surface area and is expected to separate into the MAOM (Grandy et al. 2007; Lavallee et al. 2020).
Soil organic carbon recovery rates were extremely variable across soils. Recovery for *L. tridentata* SOC was 105% (±11.7% SE) for ambient soils and 99% (±12.4% SE) for eCO₂ soils. In contrast, interspace SOC recovery was 142% (±14.5% SE) and 119% (±16.4% SE). N recovery rates were only calculated for *L. tridentata* samples because they had to be run in separately to get total N values. Recovery varied from 36-120% with a mean of 82.5% recovery for ambient and 73.5% for elevated CO₂ samples. There was a strong, positive relationship between C recovery and N recovery rates (Spearman’s rho= 0.797, p < 0.001). Samples with lower SOC concentrations tended to have higher recovery rates while high SOC resulted in low recovery. This may be attributable to SOC loss due to solubility in SPT. Throop et al (2013) found that in soils from the Chihuahua Desert, 2-3 mg of C could be solubilized in SPT. This explains low recovery rates but does not explain recovery greater than 100%.

Fig 3.1 Percent of total recovered soil organic carbon in particulate organic matter (POM) and mineral associated organic matter (MAOM) for *L. tridentata* and interspace soils at two depths (0-20 cm, 20-40 cm) in the soil profile.
On average, POM fraction contained 23.3% of recovered C and 16.6% of recovered N. MAOM contained 76.7% of C and 83.4% of N (Fig 3.1). This is consistent with the higher C:N of POM (~11-13) than MAOM (~7-8). The amount of C and N in each fraction varied slightly by cover type and treatment. The amount of C in MAOM and POM of *Larrea tridentata* soils at 0-20 cm was the same under both CO₂ treatments. At 20-40 cm there was a slight shift of the relative amount of C from POM to MAOM under eCO₂ treatment (13.6%, p= 0.06). The ratio of C in each fraction did not change under eCO₂ for interspace soils at either depth. The nitrogen pool showed a similar trend in a shift of N from POM to MAOM under eCO₂ for *L. tridentata* soils at 20-40 cm (10%, p= 0.1) and no other significant changes in the ratio of N in POM relative to MAOM.
Contrary to our expectations, $\delta^{13}C$ of POM was more enriched than MAOM in both cover types and at both depth of the soil profile (Fig 3.2). In another study of dryland shrub soil organic matter, Throop et al (2013) conducted a 7-step density fractionation and found a parabolic relationship between $\delta^{13}C$ and soil density fraction. $\delta^{13}C$ of the lightest fractions ($<1.65 \text{ g}\cdot\text{cm}^{-3}$) was around -23‰, medium densities (1.65-1.85 \text{ g}\cdot\text{cm}^{-3}) were most enriched around
-18‰, but the highest densities (> 2.65 g·cm⁻³) dropped back down to be as depleted as -28‰. We believe that in our two-pool fractionation, the MAOM fraction we isolated may dominated by this unusually depleted heaviest fraction.

The difference in δ¹³C between POM and MAOM in our study suggests that input sources of C and SOC stabilization mechanisms may be different in drylands relative to more mesic ecosystems (Waring et al. 2021). A three-pool separation, with additional isolation of a high-density fraction, might be more appropriate for these soils. Further analysis of this fraction of SOM, including ¹⁴C measurements, are needed in order to understand the origin of C in this fraction and if it has a longer turnover time than other pools of C.

Carbon and Nitrogen Stocks

Ten years of elevated atmospheric CO₂ (eCO₂) treatment resulted losses of C in both POM and MAOM for soils from L. tridentata (Fig 3.2A). In the top 20 cm of soil, MAOM-C was marginally lower by 83.1 g C·m⁻² or 26.6% (p=0.06) and there was no change in POM at 0-20 cm. At 20-40 cm, eCO₂ resulted in a decrease of L. tridentata POM-C by 72.1 gC·m² or 69.4% (p=0.05) and MAOM-C by 88.8 gC·m² or 33.3% (p= 0.048). Nitrogen stocks were less responsive to eCO₂ than C stocks for both fractions (Fig 3.2B). At 20-40 cm in depth, eCO₂ caused a 5.4 g N·m² or 67.2% decrease in L. tridentata POM-N that was only marginally significant (p= 0.07) and resulted in no change at 0-20 cm. MAOM-N of L. tridentata soils did not change under eCO₂ for either depth. Interspace soils exhibited no response to eCO₂ in C or N for both fractions at either 0-20 or 20-40 cm depth except for a marginal increase of 12.3 gN·m² in MAOM-N at 0-20 cm (p = 0.09).

Elevated CO₂ affected δ¹³C of POM and MAOM only for L. tridentata soils (Fig 3.2C).
At 20-40 cm, POM-$\delta^{13}$C decreased by 2.2‰ ($p=0.04$) and MAOM-$\delta^{13}$C decreased by 2.7‰ ($p=0.04$) while there was no effect on $\delta^{13}$C for either fraction at 0-20 cm. Interspace soils showed no response of $\delta^{13}$C to eCO$_2$. Soil $\delta^{15}$N remained largely unaffected by eCO$_2$ treatment (Fig 3.2D). The only response was in POM-$\delta^{15}$N of interspace soils which decreased by 1.7‰ under eCO$_2$ ($p=0.02$).

C:N of POM and MAOM was largely unresponsive to eCO$_2$ due to either parallel responses of C and N to treatment for some fractions or to a lack of response for either C or N (Fig 3.3). For interspace soils at 0-20 cm, POM-C:N increased from 11.3 to 13.3 ($p=0.01$) and showed a similar trend at 20-40 cm with POM-CN increasing from 12.1 to 13.7 ($p=0.06$). Despite an increase in MAOM-N for interspace soils, MAOM-C:N was not effected by eCO$_2$ for either interspace or _L. tridentata_ at either depth.

**Fig 3.3** C:N of soil organic matter from of particulate organic matter (POM; dashed blue lines) and mineral associated organic matter (MAOM; solid orange lines) soil fractions. Soils sampled represent two cover types, _L. tridentata_ and interspace soils, and two soil depths, 0-20 cm and 20-40 cm. Points represent estimated marginal means and vertical bars and standard error of the mean. There were no differences between CO$_2$ treatments.
**Pyrolysis GC/MS**

We used pyrolysis-GC/MS to assess changes in soil organic matter chemistry in the fine fraction (> 1.85 g cm\(^3\), < 20 um) of soils sampled from 0-20 cm under both interspace and *L. tridentata* cover types. Substrate chemistry can show if the microbial community preferentially targeted N rich compounds for decomposition. Due to low replication (n = 3 for each plant x CO\(_2\) treatment) and high variation among samples there were no differences by CO\(_2\) treatment for any compound class. However, trends in these samples are still useful to examine.

![Compound Source Diagram](image)

**Fig 3.4** Composition of soil organic matter in the heavy fraction smaller than <20 um as measured by pyrolysis GC/MS and presented as relative abundance. 180 compounds identified across samples were classified as being derived from one of 7 classes or categorized as unknown. There are no significant differences by treatment among classes. The majority of the lipid class consists of palmitic acid (> 50%) and there was also no treatment effect on the relative abundance of that compound.
Pyrolysis GC/MS identified a total of 180 compounds across all samples, averaging over 50 compounds per sample. The most common compound in all samples was palmitic acid (C-16) which alone represented 70% of interspace soil organic matter and 40-50% of *L. tridentata* soils. *Larrea tridentata* soils from the ambient CO$_2$ treatment had the greatest number of identified compounds at 79 (±11.5 SE) while soils from the same cover type in the eCO$_2$ treatment had 28% fewer compounds at 56.7 (±13 SE). Approximately the same number of compounds were identified for interspace soils of either CO$_2$ treatment, 50.3 (±7.33 SE) for ambient CO$_2$ and 52.5 (±16.5 SE) for eCO$_2$.

All identified compounds were classified as belonging to one of seven “source” classes, indicating from where each organic molecule is derived. Compounds without a known source were assigned as “Unknown Origin”. Lipids were the largest class for all samples due solely to the high abundance of palmitic acid. Lignin was the least abundant class of compounds, making up less than 1% of organic matter for all samples. This is due to the density separation prior to size fractionation, removing POM that is primarily derived from plant matter and is high in lignin. Compounds that were of unknown origin were less than 10% of identified molecules in interspace samples and approximately 12% of *L. tridentata* molecules. Elevated CO$_2$ had no statistically significant effects on any class of compounds for *L. tridentata* or interspace.

**DISCUSSION**

*Loss of POM and MAOM under eCO$_2$*

At the Mojave FACE site, a decrease in SOC stability is strongly related to vegetation cover. Soil from the dominant shrub cover type, *L. tridentata*, exhibited a loss of approximately 30% of MAOM in the top 40 cm of the soil profile. This is consistent with our hypothesis that eCO$_2$ can lead to SOC destabilization and adds to the growing body of evidence that MAOM the
stability of MAOM is limited under disturbance (Lavallee et al. 2018; Jilling et al. 2020). Soils at the rooting depth (20-40 cm) of *L. tridentata* also exhibited a 70% decrease in POM while showing no treatment effect in surface soils (0-20 cm). This result supports the idea that rhizodeposition can stimulate decomposition of both POM or MAOM. However, the lack of response in POM at the soil surface contradicts our hypothesis that POM would increase under eCO₂ due to an increase in aboveground litter inputs (Newingham et al. 2014). This could be an artifact of homogenizing soils from the top 20 cm rather than finer-scale resolution; it’s possible that an increase in POM at the top 5-10 cm was countered by a loss of POM from 10-20 cm as was observed at 20-40 cm. In contrast to these losses from *L. tridentata* soils, interspace soils exhibited no change in POM or MAOM at either depth. This supports our prediction that changes in SOC stability under eCO₂ are driven by changes in shrub-derived organic matter inputs.

Our results indicate that both POM and MAOM are susceptible to microbial decomposition under eCO₂ disturbance. The addition of easily mineralized organic compounds, such as litter leachates or rhizodeposition, can provide energy for microbial decomposition of old organic matter (Kuzyakov et al. 2000). The net change of SOC is a balance between accumulation of new C and “priming” of old C. One predictor of this balance is the amount of clay in the soil which is a proxy for the amount of mineral surface available to accumulate MAOM (Sulman et al. 2014). The soils at the Mojave Desert FACE are extremely low in clay (< 4%) and so it is possible that when new C is added to the system under eCO₂, mineral surfaces are already saturated and cannot stabilize the new C inputs (Six et al. 2002). Instead, this C is available for microbial metabolic processes supporting decomposition of other organic matter.
and ultimately a loss of SOC. At the Mojave FACE site, it appears that both POM and MAOM are susceptible to mineralization under these conditions.

Evidence of C limitation

The relationship between C and N for each fraction did not change as expected under eCO$_2$. POM-C and -N did not change under eCO$_2$ in surface soils, and both decreased by ~70% at the rooting depth indicating non-selective mineralization or organic matter regardless of C and N concentration. MAOM-N did not change under eCO$_2$ at either surface and rooting depths while MAOM-C decreased by ~30% throughout the profile which would indicate preferential uptake of C. Neither of these responses is consistent with our hypothesis that a decrease in SOC, particularly MAOM, would be associated with microbial mining for N (Phillips et al. 2012; Vestergård et al. 2016). Microbial mining is thought to occur when the addition of C to the system allows for the decomposition of previously inaccessible N-rich MAOM in order to liberate N for metabolic processes (Craine et al. 2007). This would result in an increase of soil C:N, which would be most likely observed as a loss of N-rich MAOM, as excess C is mineralized relative to N. In contrast to several other FACE studies, these results suggest that the Mojave Desert is C limited rather than N limited.

Microbial mining is often assumed to be the driver of SOC loss under eCO$_2$. In N limited systems, priming caused by eCO$_2$ increases N mineralization and supports greater NPP (Terrer et al. 2021). Accelerated N cycling is a consistent trend across under eCO$_2$ studies and is strongest in N-poor soils (Phillips et al. 2012; Ochoa-Hueso et al. 2014; Terrer et al. 2018). At the Mojave FACE site, however, Billings et al found that increased N cycling is accompanied by increased microbial N immobilization (Billings et al. 2002, 2004). Isotopic evidence from our study
supports the theory of N immobilization at Mojave FACE site. $\delta^{15}$N of organic matter becomes enriched as it undergoes decomposition due to the discrimination of microbial enzymatic processes against the heavier $^{15}$N (Delwiche and Steyn 1970), hence MAOM has a higher $\delta^{15}$N than POM. Assuming increased microbial activity is responsible for the observed loss of SOC under eCO$_2$, we would expect soil organic matter to become more enriched in $^{15}$N. In this study we found that neither MAOM nor POM from L. tridentata soils shows a change in $\delta^{15}$N. This is consistent with microbial immobilization of N. Without plant uptake of mineralized N, there is no net change $\delta^{15}$N of the soil organic matter pool because it includes the enriched microbial biomass N and depleted plant derived organic matter N.

These results indicate that this arid ecosystem is not primarily N limited (LeBauer and Treseder 2008; Ladwig et al. 2012) and that other limitations, such as water availability, inhibit plant growth under eCO$_2$. Previous studies have also shown nutrient limitations may shift from N limitation in the spring to C limitation in the summer and autumn and that net limitation may be dependent on precipitation patterns (Hooker and Stark 2012; Norton et al. 2012). The timing of microbial decomposition of plant C inputs as related to periods of peak plant growth may play a critical role in understanding arid ecosystem response to eCO$_2$ and should be addressed more directly in future studies.

*Belowground C flux, not aboveground, drives loss of SOC*

We hypothesized that SOC stability in surface soils (0-20 cm) would be primarily affected by the increase in aboveground shrub litter inputs at Mojave Desert FACE site. POM is derived from fragmentation of plant detritus and so we would expect this pool to increase with the greater aboveground litter inputs under eCO$_2$. Our results, however, show no change in POM
at 0-20 cm and only a marginal loss of MAOM-C at 0-20 cm indicating the change in aboveground litter inputs did not substantially affect SOC stability. *Larrea tridentata* shrubs are known to have lateral roots that are concentrated deeper in the soil profile, around 30 cm (Singh 1967; Fonteyn and Mahall 1981) and so the loss of C in both fractions at 20-40 cm is consistent with a mechanism of rhizosphere C inputs driving changes in microbial activity.

Particulate organic matter is thought to be a fast cycling fraction of C that is susceptible to rapid decomposition (Kögel-Knabner et al. 2008). While early stages of POM decomposition are dominated by leaching of dissolvable compounds, later stages require microbial enzymatic activity to decompose complex polymer structures. Therefore, water and nutrient availability can limit decomposition of POM at different stages (Hooker and Stark 2012). The lack of change in POM-C in surface soils could indicate that in this desert the POM fraction is more stable due to water limitation. Although this would seem at odds with the loss of POM-C deeper in the soil profile, there is evidence that in arid ecosystems deeper soils retain soil moisture and maintain microbial activity while shallower soils dry out more quickly inhibiting decomposition (Austin et al. 2004). Exploring the role of water limitation in preserving SOC stocks may help explain why arid ecosystems do not respond to eCO2 as would be expected for a C-limited ecosystem.

The change in POM cycling at the rooting depth also points to rhizosphere C flux as the driver of SOC decomposition. If new rhizosphere C is incorporated into POM, we would also see a decrease in δ13C due to the depleted source of CO2 in the treatment plots. Consistent with this theory, the 70% decrease in POM at 20-40 cm was accompanied by a 2.2‰ decrease in δ13C relative to control. Incorporation of new C while also yielding a net loss of C indicates a rapid turnover of the existing POM pool, possibly through the incorporation of C from fine root litter. POM-C in surface soils does not show a change in δ13C to eCO2 which indicates that new C
from aboveground litter sources is not incorporated into this pool and that the increase litter production under eCO$_2$ is not a main driver of changes in SOC at Mojave FACE.

Carbon found in the MAOM fraction of soil is considered more stable due to strong chemical bonds inhibiting microbial mineralization (Kleber et al. 2015; Cotrufo et al. 2015). It is therefore surprising that we observed not only a 33% decrease in MAOM-C under eCO$_2$, but also incorporation of new C into this fraction. At the rooting depth of *L. tridentata*, MAOM- $\delta^{13}$C was depleted by 2.7‰ relative to control. This change indicates that MAOM in these soils is an actively cycling pool of C under eCO$_2$ (Kleber et al. 2015). However, we cannot say if this pool is cycling more rapidly than under ambient CO$_2$ because we do not have a parallel tracer in the control plots. Regardless, the decrease in MAOM provides evidence that alleviation of C limitation allows microbial decomposition of this pool at a greater rate than under ambient CO$_2$. Further studies should focus on the microbial response to rhizosphere C inputs, particularly shifts in carbon use efficiency, and consider the impacts of roots on deeper soils.

*Non-selective decomposition of MAOM*

The goal of using py-GC/MS to analyze organic compounds in MAOM was to further understand shifts in compounds from different sources and to provide evidence of N-mining as a mechanism of SOC loss under eCO$_2$. Given our hypothesis that MAOM would be used by the microbial community as a source of N, we predicted that we would see a decrease in N-rich compounds. Our results did not support this hypothesis as no class of compounds showed a change in relative abundance in response to of eCO$_2$. This may be due to our low replication (N=3) and high spatial variation in organic compounds of these soils. Elevated CO$_2$ slightly increase the relative abundance of palmitic acid, which is derived from microbial biomass, for *L.*
Although the amount of microbial necromass in MAOM is also dependent on carbon use efficiency, this increase generally supports the theory that loss of MAOM-C in *L. tridentata* soils is due to an increase in microbial activity. Overall, our Py-GC/MS data do not support an N-mining hypothesis and further quantitative research is necessary to understand if changes in SOC can be attributed to changes in microbial carbon use efficiency.

**CONCLUSION**

Isolating two pools of SOC with contrasting stability yields further insight into the changes in C cycles at the Mojave FACE site. We find evidence that the pool of C considered more stable in other ecosystems, MAOM, is susceptible to mineralization under elevated CO₂. The more rapidly cycling pool of C, POM, is vulnerable to decomposition possibly due to rhizodeposition and the more favorable moisture conditions deeper in the soil profile. Finally, we find that this arid ecosystem appears to be primarily C limited as there is no evidence to support microbial N mining even under elevated mineralization conditions supported by eCO₂. Further research into the mechanisms of SOC persistence in arid ecosystems under multiple effects of climate change, such as the interactions of eCO₂ and changing precipitation regimes, is needed to understand the future trajectory of the global C cycle.
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CHAPTER FOUR

EFFECTS OF LONG-TERM ELEVATED ATMOSPHERIC CO2 ON SOIL ORGANIC CARBON MINERALIZATION AND PRIMING IN AN ARID ECOSYSTEM
ABSTRACT

The effect of elevated atmospheric CO$_2$ on carbon (C) flux to an ecosystem can impact soil organic carbon (SOC) stocks through changes in soil microbial activity. Carbon inputs to the soil in the form of simple organic molecules may stimulate decomposition of SOC through a microbial priming mechanism. Priming is thought to increase as SOC concentrations decrease, but this effect is not well studied in low SOC soils of arid ecosystems. Further, it is not known if changes in SOC driven by elevated CO$_2$ leads to progressively more or less priming. Here we explore soil C mineralization and priming in soils from the Nevada Desert Free-Air Carbon Dioxide Enrichment experiment to understand how addition of simple C compounds interacts with the effects of long-term exposure to elevated CO$_2$. We focus on soils from two major cover types of the Mojave Desert, large perennial shrubs (*Larrea tridentata*) and non-vegetated interspace using a short-term incubation experiment (168 hours) with $^{13}$C-labelled glutamic acid substrate.

We found that ten years of exposure to elevated CO$_2$ has few impacts on SOC priming with addition of simple C substrate, but that respiration is decreased in elevated CO$_2$ soils when incubated without C addition. Interspace soils are more susceptible to mineralization than *L. tridentata* soils with C addition and exhibit consistently positive priming, likely due to significant C and N limitation. In contrast, *L. tridentata* soils exhibited initial positive priming but priming becomes negative over the course of the incubation, indicating strong C limitation that is not alleviated with addition of glutamic acid. We further found that soil organic carbon concentration is closely related to cumulative priming and that dissolved organic carbon concentration is strongly related to respiration in soil incubated without C addition. This result indicates that the C:N ratio of simple C compounds entering the soil and soil moisture availability may control priming in shrub dominated soils. Other global change impacts, such as
encroachment of annual herbaceous species in deserts or changes in precipitation regimes, may drive high priming in interspace areas. Understanding the temporal relationship between C addition and soil moisture availability in deserts is critical to predicting impacts of elevated CO$_2$ on SOC stocks and global C cycling.

**INTRODUCTION**

Rising atmospheric CO$_2$ concentration is perturbing the global carbon (C) cycle due in part to effects on terrestrial ecosystems processes. Two major processes affected are net primary productivity (NPP) and heterotrophic respiration ($R_{H}$) (Carney et al. 2007; Dawes et al. 2013; Crowther et al. 2016; Carrillo et al. 2018; Jiang et al. 2020). These two fluxes relate the pools of inorganic C in the atmosphere (CO$_2$) to other stocks where C is sequestered in organic forms, such as vegetation and soils (Schlesinger 1977; Jackson et al. 2017). In recent decades, a rise in $R_H$ relative to total soil respiration indicates an acceleration of the flux of C moving from soils to the atmosphere via decomposition of soil organic carbon (SOC) (Bond-Lamberty et al. 2018). When disturbances associated with climate change accelerate decomposition of SOC beyond NPP inputs there is a net increase of atmospheric CO$_2$. Understanding how elevated atmospheric CO$_2$ impacts SOC decomposition is critical as any changes that increases the flux of CO$_2$ out of sequestered C stocks may accelerate future climate change (Todd-Brown et al. 2014; Crowther et al. 2016).

Elevated CO$_2$ (eCO$_2$) impacts terrestrial C cycling by increasing rates of photosynthesis and therefore the flux of organic C into an ecosystem (Norby et al. 2005; Carney et al. 2007; Hungate et al. 2009). The impact of this C flux on soil SOC stocks is dependent on C allocation, whether a plant invests that C in new aboveground growth, root growth, or other rhizosphere C flux (Schimel et al. 2015; Sokol and Bradford 2019; Terrer et al. 2021). However, our
understanding of this relationship and impacts on SOC stocks has high uncertainty (Le Quéré et al. 2009; Friedlingstein et al. 2020). An increase in low molecular weight organic compounds, such as root exudates or litter leachate, has complex impacts on SOC stocks that depend on ecosystem properties including nutrient limitation and edaphic properties such as soil clay content (Sulman et al. 2014). These compounds are easily assimilated by the microbial community for use in metabolic processes, ultimately being respired or catabolized into microbial biomass and byproducts (Geyer et al. 2016; Malik et al. 2020). Under some circumstances, however, the addition of simple C to the soil stimulates or suppresses microbial mineralization of other C in the soil, the pre-existing SOC stocks (Bingeman et al. 1953). The effect of C added on SOC mineralized is called “priming” and can be positive or negative depending on if mineralization is increased or decreased with the added substrate (Kuzyakov et al. 2000). The addition of new simple C caused by eCO₂ can therefore impact the size and stability of the SOC pool through the microbial priming response.

Soil organic carbon concentration is a primary control on the priming effect; soils with higher SOC show negative priming while low SOC soils tend to show positive priming (Carney et al. 2007; Bastida et al. 2019). This implies that disturbances that change SOC stocks can affect future susceptibility to priming. Elevated CO₂ drives changes to both the composition and quantity of soil organic matter and so may have an interactive effect of how microbial priming changes after long term exposure to eCO₂ (Phillips et al. 2012; Sulman et al. 2014; Fang et al. 2015). A decrease in SOC under eCO₂ could create a positive feedback, as lower SOC results in greater priming. Alternatively, continued inputs of simple C may have diminishing effects on microbial priming, particularly if under C added conditions some other nutrient becomes limiting to decomposition. The cumulative impacts of eCO₂ derived C inputs and the resulting microbial
priming response may change not only the size of the SOC pool, but the stability of SOC to further mineralization. Understanding how eCO$_2$ affects the susceptibility of SOC stocks to priming is key to predicting vulnerability of SOC stocks, and the relationship to atmospheric CO$_2$ concentrations, under future climate scenarios.

Arid ecosystem soils have low organic matter content and are predicted to be vulnerable to high rates of positive priming (Bastida et al. 2019). However, unlike more temperate ecosystems, deserts are thought to be primarily C limited (Billings et al. 2004; Schaeffer et al. 2007). Soil C cycling depends on not only organic matter inputs, but also the timing of precipitation events and effects of vegetation cover (Austin 2011; Hooker and Stark 2012; Norton et al. 2012). The interaction of these variables may disrupt the expected priming response in desert ecosystems and how eCO$_2$ changes that response. Increased C flux in desert soils is primarily concentrated in the vicinity of large, perennial shrubs (Schlesinger et al. 1996). These soils experience greater litter inputs and rhizosphere C flux than unvegetated soils and have higher concentrations of SOC relative to unvegetated spaces between shrubs (Vinton and Burke 1995). Therefore, we would expect to see greater impacts of eCO$_2$ stimulated C inputs, and consequently greater changes in SOC stability, near large shrubs.

While shrub affected soils are important to understand because of their predicted larger response to eCO$_2$, unvegetated soils can make up a greater amount of cover than perennial shrubs and are therefore critical to understanding ecosystem level C cycling. The difference in C inputs between these two microsites, shrub and interspace soils, also create different legacies of C flux. Shrubs experience both aboveground inputs from litter and belowground rhizosphere C flux while interspace soils have limited aboveground inputs but can experiences rhizosphere C flux from neighboring shrub root systems. The stability of the existing SOC pool is dependent on this
history of C inputs to the soil and so shrub and interspace soils likely experience distinct SOC stabilization (Sokol and Bradford 2019). Comparing these two distinct microsites can help us understand how changes in above and belowground C flux stimulated by eCO$_2$ differentially affect SOC mineralization.

Here, we use soils from the Mojave Desert Free-Air Carbon dioxide Enrichment experiment in a short-term incubation study to explore susceptibility of SOC to microbial mineralization in arid soils. These soils were harvested after 10 years of exposure to eCO$_2$ at the end of the growing season. Soils were collected to 20 cm in depth from below the canopy of the major shrub cover type, *Larrea tridentata*, and from unvegetated “interspace” soils. Previous results show that soils under *L. tridentata* exhibited a loss of SOC stock from 0-100 cm while interspace soils exhibited no change in eCO$_2$ (Jensen et al. Chapter 2). Soils from ambient CO$_2$ treatment will allow us to understand the susceptibility of SOC to mineralization and priming under non-disturbed conditions while soils from the eCO$_2$ plots will allow us to explore the legacy of this treatment on SOC stability to mineralization.

In this study we quantified respiration in soils amended with water or with a glutamic acid solution throughout an 8-day incubation. By using a $^{13}$C-labelled glutamic acid substrate we calculated soil priming as the difference in soil derived CO$_2$ from the soils receiving glutamic acid solution amendment and those receiving only water. We also compare soil respiration rate and cumulative primed C to SOC and dissolved organic C (DOC) concentrations to understand the influence of these pools on microbial activity. We aim to address three main questions with this experiment: 1) Does elevated CO$_2$ alter the susceptibility of SOC to overall mineralization? 2) Does 10-years of elevated CO$_2$ make soils more or less susceptible to priming? And 3) Does perennial shrub cover have an interactive effect on susceptibility to mineralization? We predict
that soils that have been previously exposed to elevated CO\textsubscript{2} will be relatively less susceptible to further mineralization and exhibit decreased positive priming. We hypothesize that \textit{L. tridentata} soils will have lower rates of priming and mineralization relative to interspace soils due to a history of belowground C flux from shrub roots and that eCO\textsubscript{2} effects will only be observed in \textit{L. tridentata} soils.

\textbf{METHODS}

\textit{Site Description}

The Nevada Desert FACE Facility (NDFF) is located in the northern Mojave Desert in the Great Basin of the Basin and Range physiographic province on the US Department of Energy’s Nevada Test Site in southern Nevada, USA. The arid climate is characterized by limited precipitation (mean annual precipitation=140 mm/yr), low humidity, and large diurnal temperature fluctuations(Jordan et al. 1999; Soper et al. 2017). Soils are Aridisols formed in calcareous alluvial fans resulting in highly variable coarse contents and textures across the site and throughout the profile, but generally ranging from loamy sands in the A horizons (0-.16 m) to coarse sands with depth (Jordan et al. 1999). There is no caliche layer such that soils are well drained and pH is in the range of 8-9 at all depths (Jordan et al. 1999).

Vegetation of the NDFF is an open, desert scrub formation typical of the Mojave Desert at mid- to low-elevations (Smith et al. 1997). The dominant vegetation at the NDFF is the long-lived evergreen shrub \textit{Larrea tridentata}, followed by the drought-deciduous shrubs \textit{Lycium andersonii}, \textit{Lycium pallidum}, and \textit{Ambrosia dumosa} (Jordan et al. 1999). Collectively, these four species comprise >70\% of the shrub population (Jordan et al. 1999). Perennial grasses, primarily \textit{Pleuraphis rigida} also make up an important part of the community. Plant interspaces represent ~80\% of the land cover and are predominately occupied by biological soil crusts.
(Webb et al. 2003). The Mojave Desert receives low annual precipitation that can be sporadic throughout the winter months such that water is the primary limitation to plant growth (Turner and Randall 1989).

Decommissioning of the NDFF after a decade of exposure to FACE provided the opportunity for the destructive sampling of an otherwise intact and undisturbed Mojave Desert ecosystem for the evaluation of the response of soils to eCO$_2$. The facility was comprised of 9, 23-m diameter circular plots (referred to as “rings” throughout): three ambient CO$_2$, three elevated CO$_2$ and three control plots to ensure CO$_2$ blowers did not affect results. Treatment was started in April of 1997 at 550 ppm CO$_2$ and was terminated in June of 2007. Due to unusually low winter precipitation in 2006-2007, all rings were irrigated in March 2007 (30 mm) to stimulate biomass growth before ceasing CO$_2$ treatment. All aboveground biomass and select belowground biomass samples were harvested at the end of the 10-year experiment (Newingham et al. 2013).

Sample Collection

During decommissioning of the NDFF, soil samples were collected following the removal of biological soil crusts and vegetation from the site. Mineral soils were sampled to 20 cm from areas previously beneath the canopy of *L. tridentata* plants as well as from the unvegetated interspace. Samples were air-dried and passed through a 2 mm sieve to eliminate coarse fragments. Soils were transported to Ithaca, NY and stored in watertight containers until analyses. No interspace samples from ring 2 (elevated CO$_2$ plot) could be located at the time of this experiment and so duplicate samples from ring 3 were substituted to maintain N = 9 for each CO$_2$ treatment.
Water Holding Capacity:

Water holding capacity was determined by weighing 5 g of dry soil into a paper filter (Whatman No. 1) in a plastic funnel. DI water was added until water began dripping through the bottom of the funnel and then allowed to drain. Funnels were covered in parafilm overnight and weighed after 24 hours. WHC was calculated as:

\[ \text{WHC} = \text{Weight wet soil} - \text{Weight dry soil} \ (g) \]

Dissolved Organic Carbon

DOC was measured using a K$_2$SO$_4$ extraction. Ten grams of each soil sample were weighed into 50 mL centrifuge tubes and forty mL of 0.05 M K$_2$SO$_4$ was added. Two centrifuge tubes without soil were used as blanks for each set of samples extracted. All samples were mixed on an orbital shaker at 150 rev min$^{-1}$ for 4 hours and allowed to settle for 15 minutes. The top 25 mL of supernatant was decanted into paper filters (Whatman No. 1) in funnels and filtrate collected in scintillation vials. Extracts were dried at 60°C until only salt crystals remained in vial. Vials were weighted and the dried material homogenized before analysis for %C and δ$^{13}$C.

Soil organic carbon

Prior to measuring soil organic carbon analysis, all soil samples were pretreated for inorganic C removal using an acid fumigation method. Soils were weighed at 20 mg directly into silver capsules on a microbalance (Sartorius Cubis, Germany) and placed in a 96 well plate. Approximately 10 uL of DI water was added to each sample. The well-plate was then placed in a vacuum desiccator along with two open 100 mL beakers of 12 M HCl. The desiccator was placed under vacuum using a hand-held vacuum pump and then closed. Samples were fumigated.
for 24 hours. After removal from the desiccator, well-plates were dried at 60°C for 24 hours, then double wrapped in tin before dry combustion.

**Pre-incubation**

All samples were pre-incubated at 10% WHC in the specimen cups that would be used for the 8-day incubation. Dry soils were weighed into specimen cups and DI water added to achieve 10% WHC for each particular soil. Soils were mixed briefly with a spatula and then capped and placed in a 22°C, dark incubation room for 5 days. This duration was determined prior to the experiment by testing different incubation times and measuring respiration rate. Five days allows for the pulse of respiration associated with water amendment and microbial disturbance to subside and achieve equilibrium.

**Incubations**

This method is based on the $^{13}$C-glucose tracing method described in Jilling et al 2021 (Jilling et al. 2021). Briefly, soil incubations are run in parallel, one with amended with water and another with a $^{13}$C-labeled substrate, and headspace CO$_2$ gas samples are collected to quantify respiration rate and $\delta^{13}$C of CO$_2$ over a multi-day incubation. We use glutamic acid as the substrate because it can be directly taken up by microbes and is representative of compounds that may be found in rhizodeposition (Morley and Baggs 2010). Universally labeled 99 AT% $^{13}$C-glutamic acid was diluted with $^{12}$C-glutamic acid to achieve ~26‰ solution, equivalent to approximately 20% once diluted by pre-incubation soil moisture. The concentration of the amendment solution was approximately 0.30 mg glutamic acid/mL to achieve the target C concentration of 10 ug C per g soil for incubation. Pre-incubated specimen cups of 25 g of soil
were amended with enough DI water or glutamic acid solution to bring samples up to 40% WHC. Soils were mixed with a spatula briefly after amending to ensure even distribution of substrate throughout soil.

Once amended, specimen cups were placed, open, inside 500 mL mason jars and capped. Caps were fitted with rubber septa for respiration measurements and gas samples were collected by taking a 15 mL headspace sample with a syringe and injecting into an evacuated 12 mL gas bench vial. Sampling was conducted at 4, 8, 12, 24, 48, 72, 120, and 168 hours. Background samples (T₀) were taken immediately after capping and then a second time point (T₁) was taken at the appropriate time interval. Between each time interval, jars were left open in a fume hood for 20-30 minutes to flush any accumulated CO₂. Preliminary trials showed this method to sufficiently drop CO₂ down to ambient concentrations prior to capping again for the next time point. Gas samples were measured for CO₂ concentration and δ¹³C-CO₂ on an IRMS coupled to a gas bench.

**Sample Analysis**

All samples (SOC, DOC, and CO₂) were measured using a continuous flow isotope ratio mass spectrometer (IR-MS; Model V Advantage; Thermo Scientific, Bremen, Germany) coupled to either an elemental analyzer (SOC & DOC) (Thermo Finnigan Carlo Erba NC2500) or gas bench (CO₂) (Gas Bench II, Thermo Scientific, Bremen, Germany)). Isotope ratios are expressed as δ values (per mil):

\[ \delta^{13}C = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000(\%) \]
where $R_{\text{sample}}$ and $R_{\text{standard}}$ are the ratios of heavy to light isotope of the sample relative to the international standards for C, Vienna-Pee Dee Belemnite. All isotopic analyses were completed at the Cornell University Stable Isotope Laboratory (COIL).

**Statistical analyses**

All statistical analyses were conducted using the statistics package R, version 4.1.0 ([http://cran.r-project.org](http://cran.r-project.org)). Effects of CO$_2$ treatment, glutamic acid amendment, and plant cover type, and incubation time on respiration, mineralization, and priming rates were assessed using a linear mixed effects model using the functions “lmer” from the “lme4” package (Bates et al 2015). The fixed effects in the model were ‘CO$_2$ Treatment’, ‘Amendment’, ‘Plant’, and ‘Time’ as a factor. The ‘Amendment’ term was removed when priming responses were analyzed as they are derived from the difference between ‘control’ and ‘glutamic acid’ amendments. All interactions were allowed between fixed effects. Random effects were ‘Plot’, ‘Sample ID’, and ‘Jar’ to account for the effect of subsampling from the same mason jar at multiple time points in throughout the incubation. Residuals were inspected for assumptions of normality required for parametric tests and data were square root transformed where necessary. Type III test of fixed effects were assessed with Satterthwaite approximation of degrees of freedom using the “lmerTest” package (Kuznetsova et al. 2017). Post-hoc pairwise comparisons were conducted using the R package “emmeans” (Lenth 2020). Significance was set at alpha = 0.05.

The amount of glutamic acid C delivered to each soil was dependent on water holding capacity which varied significantly by cover type such that *Larrea tridentata* soils received on average 52.1 ± 5.9 ug glutamic acid C *g soil$^{-1}$ while interspace soils received 36.8 ± 5.5 µg glutamic acid C *g soil$^{-1}$. To account for this effect, we added glutamic acid C (μg C * g soil$^{-1}$)
as a covariate when developing the model. We found that glutamic acid C did not have main or interactive effects on absolute respiration (F=53.4, p = 0.17) or on respiration rate (F= 0.087, p= 0.77) and so was not included in the final model.

Cumulative respiration and cumulative priming were analyzed separately at 4 hours and 168 hours to compare the two distinct phases of mineralization- initial pulse response and multiday incubation. Arid soils are rarely, if ever, wet for multiple days and so 168 hour is not ecologically realistic for these soils. Here we use it as a means to compare total maximum SOC mineralizability. 168 hours was chosen so as to capture the full effects of an input of C (glutamic acid addition) while minimizing the amount of respiration from microbial biomass turnover (ie microbes taking up the $^{13}$C label into biomass and then eventual mineralization of that biomass).

The data was subset by each time point and the linear mixed effects models were the same as described for respiration rate except without the fixed effect of ‘Time’ and without the random effect of ‘Jar’.

Soil organic carbon and dissolved organic carbon pools were not manipulated in this experiment but varied naturally across samples. We used this natural variation to explore the relationship between these two pools of C and respiration responses. We fit a series of secondary linear mixed effects models where ‘SOC’ (mg C * g soil$^{-1}$) or ‘DOC’ (µg C * g soil$^{-1}$) were included instead of ‘plant’ as fixed effects, in addition to the fixed and random effects described previously. Cover type (‘plant’) is used as a proxy for a suite of characteristics that vary between shrub covered soils ($L$. tridentata) and un-vegetated soils (interspace), such as SOC and DOC which are expected to be higher under shrubs. By replacing the ‘plant’ term with SOC and DOC we can explore the impact of these specific soil characteristics independent of cover type designation. Spearman rank correlation from the ‘stats’ package (R Core Team) was used as
post-hoc assessment. We removed one sample from *L. tridentata* for post-hoc assessment that had an SOC concentration more than 2 s.d. above the mean.

**RESULTS**

*Soil characteristics*

Soil organic carbon (mg C*g soil$^{-1}$) varied by cover type (F= 80.6, p < 0.001) but not by CO$_2$ treatment (F=0.15, p = 0.70) (Fig 4.1). There was no interaction between treatment and cover type for SOC (F = 0.45, p = 0.5). Soil organic C concentration was more than two times greater in *L. tridentata* soils (3.31 ± 0.09 mg SOC*g soil$^{-1}$) compared to interspace soils (1.49 ± 0.2 mg SOC*g soil$^{-1}$). Dissolved organic carbon concentrations also varied by cover type (F = 10.3, p = 0.002) and did not have a main effect of CO$_2$ treatment (F= 3.7, p= 0.11) (Supplemental Fig 2). However, there was a significant cover type* CO$_2$ treatment effect (F= 5.6, p= 0.021). DOC of interspace soils averaged 36.3 (±5.00) µg DOC *g soil$^{-1}$ and did not vary by CO$_2$ treatment (p = 0.55). *Larrea tridentata* soils did vary by CO$_2$ treatment (p = 0.021) with 67.6 (±7.44) µg DOC *g soil$^{-1}$ in ambient CO$_2$ soils and 37.4 (± 7.19) µg DOC *g soil$^{-1}$ in eCO$_2$ soils (Fig 4.2).
Fig 4.1 Soil organic carbon concentrations (mg C·g soil⁻¹) for soils used in the incubations of this study. Soils are from two cover types (interspace and *L. tridentata*) and ambient and elevated CO₂ treatments at the Nevada Desert FACE Facility.

Fig 4.2 Dissolved organic carbon concentrations (µg C·g soil⁻¹) for soils used in the incubations of this study. Soils are from two cover types (interspace and *L. tridentata*) and ambient and elevated CO₂ treatments at the Nevada Desert FACE Facility.
Soil respiration

Glutamic acid amendment had the expected effect of increasing initial soil respiration rates, with peak rates occurring at our first time point (4 hours), and then decreasing over time to the level of control samples (Fig 4.3). In contrast, control amendment soils showed a lag in respiration, peaking around 12 hours for all cover type by CO$_2$ treatment combinations. For *L. tridentata* soils amended with glutamic acid, respiration rates returned to the level of control.
rates by 72 hours while interspace soils amended with glutamic acid remained at an elevated rate of respiration until 120 hours. In general, _L. tridentata_ respiration rates were greater than interspace. Elevated CO₂ affected respiration rates only in _L. tridentata_ soils. Elevated CO₂ _Larrea tridentata_ soils amended with water exhibited a 69.3% decrease in respiration rate at 4 hours, going from 11.3 µg CO₂-C•hr⁻¹ in ambient CO₂ soils to 3.4 µg CO₂-C•hr⁻¹ in elevated CO₂ soils (p < 0.001). In soils receiving glutamic acid addition, respiration rates were 22.2% lower in eCO₂ soils at 12 hours (p= 0.004). Respiration rates did not vary by CO₂ treatment in interspace soils at any time point.

Cumulative soil derived CO₂-C did not show consistent trends with eCO₂ treatment. _Larrea tridentata_ soils amended with water showed higher cumulative respiration in ambient
CO₂ soils for the first 12 hours of incubation but this was the only difference for eCO₂ treatment for either cover type. We focused on cumulative respiration at 4 hours and 168 hours to understand the effects of cover type, amendment, and eCO₂ treatment on initial and total respiration responses (Fig 4.4). Both time points had significant main effects for cover type (p < 0.01) and amendment (p < 0.001) but no main effect for treatment (p > 0.05). However, at the 4-hour time point in *L. tridentata* soils amended with water, there was more soil C respired from ambient CO₂ soils (43.9 ± 12.7 µg C) than from eCO₂ soils (14.0 ± 6.7 µg C) (p=0.038).

Interspace soils amended with water and neither cover type when amended with glutamic acid showed a CO₂ treatment effect on soil derived C respired. At the end of the 168-hour incubation, *L. tridentata* soils showed slightly, although not statistically significant, more cumulative respired C in ambient than eCO₂ soils for both amendments (control p = 0.2, glutamic acid p = 0.13) while interspace samples showed no trend of difference between CO₂ treatments.

Mineralization rate was calculated by dividing soil derived respiration rate by soil organic carbon concentration of soils (CO₂-C • mg SOC⁻¹ • hr⁻¹). As with soil derived respiration, there was no main effect of treatment (F= 0.14, p = 0.73). In contrast, mineralization rate did not vary by cover type, indicating that SOC concentration is a strong control on cumulative respiration in these soils (F= 1.33, p = 0.26). There was a significant interaction of treatment and time (F = 2.2, p = 0.03) and a marginally significant four-way interaction of plant, treatment, amendment, and time (F= 1.97, p = 0.057). Pairwise comparisons showed that mineralization rate differed by CO₂ treatment at 4 hours for *L. tridentata* soils amended with water (p= 0.006) where mineralization rate was almost 3x higher in ambient CO₂ soils (3.1 ± 0.7 µg C • mg SOC⁻¹*hr⁻¹) than eCO₂ soils (1.1 ± 0.3 µg C • mg SOC⁻¹*hr⁻¹). Mineralization was also greater in ambient CO₂ at 12 hours in *L. tridentata* soils with glutamic acid amendment (3.8 ± 0.8 µg C • mg SOC⁻¹*hr⁻¹) than eCO₂
soils (1.8 ± 0.4 μg C • mg SOC−1*hr−1) (p= 0.025).

Soil Priming

Priming rate varied by cover type (F =8.9, p= 0.006) and time (F= 75.2, p < 0.001) with a significant interaction between cover type and time (F = 3.7, p = 0.001) but no effect of CO₂ treatment (F= 0.02, p= 0.88) (Fig 4.5). The rate of primed C was highest for both cover types at 4 hours and declined rapidly after the first sampling time point. *Larrea tridentata* soils started with a higher priming rate than interspace soils but by 12 hours priming in *L. tridentata* soils became negative and remained at or below 0 for the remainder of the incubation. Interspace soils exhibited low but positive priming rates for the entire incubation.

![Graph showing rate of priming by cover type and CO₂ treatment](image)

4.5 Rate of priming (μg C primed • hr⁻¹) by cover type and CO₂ treatment. There were no differences in priming rate by CO₂ treatment (F= 0.02, p= 0.88) by difference by cover type (F =8.9, p= 0.006), (F= 75.2, p < 0.001), and an interaction between cover type and time (F = 3.7, p = 0.001). Although *L. tridentata* soils started with the highest priming rate, by 12 hours priming rate became negative while interspace soils exhibited positive priming throughout the incubation.
Net cumulative priming was analyzed at 4 hour and 168 hours independently. Cumulative priming varied by cover type at 4 hours (F= 12.0, p = 0.002) and at 168 hours (F= 4.2, p = 0.049) (Fig 4.6). There was no CO₂ treatment effect on net cumulative priming and no interactive effect of cover type and CO₂ treatment at either time point. Net cumulative priming at 4 hours was about 50% greater in *L. tridentata* soils (87.3 µg ± 11.6 µg C) relative to interspace soils (45.9 µg ± 8.0 µg C). At the end of the 168-hour incubation, net cumulative priming for *L. tridentata* was negative (-86.8 ± 54.7 µg C for ambient CO₂ soils and -141.4 ± 62.9 µg C for eCO₂ soils) indicating that cumulative soil derived respiration was greater in soils receiving water relative to soils receiving glutamic acid amendment. In contrast, interspace soils exhibited positive net cumulative priming (186.1 ± 39.0 µg for ambient CO₂ soils and 229.7 ± 49.8 for eCO₂ soils).

![Fig 4.6 Net cumulative primed C (µg C) after 4 hours and 168 hours of incubation. Cumulative priming varied by cover type at both time points (p < 0.05) and there was no effect of CO₂ treatment and no interactive effect at either time point. Negative priming is due to higher respiration in control (water) soils relative to glutamic acid amended soils.](image)
Priming effect was calculated as the ratio of primed C to the amount of C respired from water control. Priming effect was only analyzed at 4 hours where priming rates were highest because low or 0 control respiration rates can result in extremely high or “infinite” (when dividing by zero) priming effects. Eleven samples were infinite or more than 2 s.d. above the mean and were excluded from analysis. There was no main effect of CO$_2$ treatment ($F = 0.003$, $p = 0.95$) or cover type ($F = 0.07$, $p = 0.79$) but there was a significant interaction of CO$_2$ treatment and cover type ($F = 5.69$, $p = 0.027$) (Fig 4.7). There was high variation in priming effect but post-hoc comparisons showed that for *L. tridentata* soils, eCO$_2$ soils had a marginally greater priming effect (5.05 ± 1.5) than ambient CO$_2$ soils (1.84 ± 0.9) ($p = 0.09$). Interspace soils showed the opposite, although not statistically significant trend of higher priming effect in ambient CO$_2$ soils (4.53 ± 1.7) relative to eCO$_2$ soils (1.64 ± 1.0).

![Fig 4.7](image)

Fig 4.7 Priming effect at 4 hours of incubation by cover type and CO$_2$ treatment. Priming effect is calculated as the ratio of primed C to C respired from water control. Priming effects > 100 were removed as they are caused by control respiration values close to zero. There is no main effect of cover type ($F = 0.07$, $p = 0.79$) or CO$_2$ treatment ($F = 0.003$, $p = 0.95$) on priming effect but there is a significant interaction between cover type and CO$_2$ treatment ($F = 5.69$, $p = 0.027$). “*” indicates $p < 0.1$ in post-hoc pairwise comparison.
**Effects of SOC and DOC on Decomposition**

Soil organic C is positively related to soil derived C respiration rate ($F=11.2, p = 0.003$) as was amendment ($F= 36.4, p < 0.001$) but not CO$_2$ treatment ($F= 0.68, p= 0.42$) (Table 4.1; Fig 4.8). Dissolved organic carbon wis also positively related to soil derived respiration rate, with main effects for DOC ($F = 24.0, p < 0.001$), CO$_2$ treatment ($F= 4.97, p = 0.034$), amendment ($F = 22.8, p < 0.001$), and time ($F= 3.53, p = 0.001$) when DOC is included in this model. This indicates that DOC explains more variation in respiration rate due to eCO$_2$ than using cover type or SOC as a predictor (Table 4.1; Fig 4.9).

Table 4.1 ANOVA of a linear mixed effects model of respiration rate ($\mu g$ CO$_2$-C * hr$^{-1}$) at A) 4 hours and B) 168 hours with fixed effects of CO$_2$ treatment, amendment, and either cover type, soil organic carbon concentration, or dissolved organic carbon concentration.

<table>
<thead>
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<th>A. 4 Hours</th>
<th>Cover Type</th>
<th>SOC</th>
<th>DOC</th>
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<tbody>
<tr>
<td>Variable</td>
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<td>F Value</td>
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<tr>
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<tr>
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<tr>
<td>Amendment</td>
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<tr>
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<tr>
<td>Treatment * Amendment</td>
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<td>Cover Type * Treatment * Amendment</td>
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<th>B. 168 Hours</th>
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<th>SOC</th>
<th>DOC</th>
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<td>Prob (F)</td>
<td>F Value</td>
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<td>12.8</td>
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<tr>
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<tr>
<td>Amendment</td>
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<td>0.04</td>
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Fig 4.8 Correlation of soil derived respiration rate (µg CO₂-C * hr⁻¹) with soil organic carbon concentration at 4 and 168 hours across both cover types. One sample with SOC greater than 2 S.D. above the mean was eliminated for this analysis. Best fit line represents significant relationships according to linear mixed model. Statistics are from linear regression.

Fig 4.9 Correlation of soil derived respiration rate (µg CO₂-C * hr⁻¹) with dissolved organic carbon concentrations at 4 and 168 hours across both cover types. Best fit line represents significant relationships according to linear mixed model. Statistics are from linear regression.
SOC concentration is not related to priming rate at 4 hours of incubation and is negatively related to priming rate at 168 hours (Table 4.2; Fig 4.10). There is a main effect of CO₂ treatment on the relationship between SOC concentration and priming rate with eCO₂ priming rates being more strongly negative than priming rates from ambient CO₂ soils. Cumulative priming was positively related to SOC concentration at 4 hours and negatively related at 168 hours with no effect of CO₂ treatment (Table 4.3; Fig 4.11). DOC was not related to priming rate or to cumulative priming at either 4 hours or 168 hours of incubation (Table 4.2 & 4.3).

Table 4.2 ANOVA of a linear mixed effects model of priming rate (µg CO₂-C * hr⁻¹) at A) 4 hours and B) 168 hours with fixed effects of CO₂ treatment, amendment, and either cover type, soil organic carbon concentration, or dissolved organic carbon concentration.

<table>
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<th>DOC</th>
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<td>Cover Type/DOC/SOC</td>
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<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>CO₂ Treatment</td>
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<td></td>
<td>Cover Type* CO₂ Treatment</td>
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Fig 4.10 Soil organic C is not related to priming rate (µg CO₂-C * hr⁻¹) at 4 hours but is at 168 hours. There is a stronger negative relationship between SOC and priming rate in soils from elevated CO₂. DOC is not related to priming rate at either time point. Best fit line represents significant relationships according to linear mixed model. Statistics are from linear regression.

Table 4.3 ANOVA of a linear mixed effects model of cumulative primed C (µg CO₂-C) at A) 4 hours and B) 168 hours with fixed effects of CO₂ treatment, amendment, and either cover type, soil organic carbon concentration, or dissolved organic carbon concentration.

<table>
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<tr>
<th>A. 4 Hours</th>
<th>Cover Type</th>
<th>SOC</th>
<th>DOC</th>
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<td>Variable</td>
<td>F Value</td>
<td>Prob (&gt;F)</td>
<td>F Value</td>
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<tr>
<td>Cover Type/DOC/SOC</td>
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<td>0.004</td>
<td>19.0</td>
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<tr>
<td>CO₂ Treatment</td>
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<td>Cover Type* CO₂ Treatment</td>
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<td>0.93</td>
<td>1.1</td>
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<table>
<thead>
<tr>
<th>B. 168 Hours</th>
<th>Cover Type</th>
<th>SOC</th>
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<td>Cover Type/DOC/SOC</td>
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<td>17.4</td>
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<td>CO₂ Treatment</td>
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<td>0.43</td>
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<td>Cover Type* CO₂ Treatment</td>
<td>0.29</td>
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DISCUSSION

This study of soils from the Mojave FACE experiment reveals patterns of soil organic carbon mineralization and priming that further our understanding of soil C cycling in arid ecosystems. The Mojave FACE site presents a unique system in which to explore the interaction of elevated atmospheric CO$_2$ and SOC mineralization in soils with known C limitation (Schaeffer et al. 2003, 2007). We found that within the first 4 hours of our incubation, *L. tridentata* soils exposed to eCO$_2$ had a priming effect more than 2x greater than soils from ambient CO$_2$ (Fig 4.7). This was contrary to our hypothesis that eCO$_2$ would result in less susceptibility to priming. However, this difference in priming effect arises from respiration in
water amended controls rather than in soils amended with glutamic acid and there was no eCO\textsubscript{2} effect on the absolute amount of cumulative primed C (Fig 4.6). Taken together these results indicate that eCO\textsubscript{2} changes both the quantity and quality of organic substrates in a way that impacts the mineralizability of SOC in both control and added C conditions.

Understanding the differences in SOC mineralization between \textit{L. tridentata} and interspace soils shows controls on priming in this system that can help us understand how C inputs from eCO\textsubscript{2} may affect SOC stocks. In general, interspace soils had lower respiration rates but higher mineralization rates (Fig 4.3). This is consistent with our hypothesis that soils from under shrub cover will be less mineralizable due to a history of C flux associated with rhizosphere C and litter inputs. Microbial transformation of organic matter results in more stable forms of SOC associated with microbial biomass and byproducts (Kögel-Knabner et al. 2008; Lehmann and Kleber 2015) while SOC from interspace soils is less processed and more susceptible to mineralization (Christensen 2001; Cotrufo et al. 2015). This result indicates that continued C inputs associated with islands of fertility create fundamentally different pools of SOC that likely experience different limitations on C cycling.

In the first 12 hours of incubation, eCO\textsubscript{2} decreased respiration (rate and cumulative C) from \textit{Larrea tridentata} soils amended with water while soils amended with glutamic acid showed no eCO\textsubscript{2} effects on respiration (Fig 2). Interspace soils exhibited no differences in respiration due to eCO\textsubscript{2}. Other studies on arid ecosystem soils show that soil moisture alone is a strong predictor of soil respiration (Saetre and Stark 2005; Hooker and Stark 2012; Norton et al. 2012). Arid ecosystem soils accumulate organic substrates during periods of low soil moisture that are rapidly mineralized upon rewetting indicating a primary water limitation in these ecosystems (Birch 1958). The lower respiration in eCO\textsubscript{2} soils with water addition indicates that
eCO2 affects decreases either the quantity or quality of this accumulated organic matter in *L. tridentata* but not interspace soils.

Adding a simple C compound to these soils dampened the effect of eCO2 on soil respiration that had been observed in water amended *L. tridentata* soils. In soils amended with glutamic acid, there was a slight decrease in respiration rate at the 12-hour time point for eCO2 soils but no difference in cumulative respired C at any time point (Fig 1). Addition of an available C substrate overwhelms the difference in available C that drives the eCO2 effect on respiration in water amended soils. This implies that eCO2 stimulated C flux affects the pool of available C available for microbial mineralization, the addition of the C itself does not result in differences to microbial respiration. This could point to a temporal separation between the effect on available SOC pool and pulses of microbial activity that change the SOC pool (Schimel and Parton 1986; Austin 2011). The increase in photosynthesis driven by eCO2 may occur during periods of high moisture, but by the time that C is reaches the soil there is not enough soil moisture for microbial activity. This scenario highlights that our incubation experiment with ample water availability for multiple days is not realistic to the Mojave Desert ecosystem and that water availability dominates the controls on microbial mineralization.

Although there were no differences in the absolute amount of C primed, the priming effect (priming relative to the respiration from control amendment samples) in ambient CO2 soils was slightly higher than in eCO2 soils at the time point with the highest priming rate (4 hours) (Fig 4.5 & 4.6). Further, we found that in the short term (4 hours), cumulative primed C is positively related to SOC but that over longer periods of time (168 hours), this relationship becomes negative (Fig 4.11). This contradicts the idea that priming is negatively related to SOC (Carney et al. 2007; Bastida et al. 2019) and instead points to a more complicated relationship.
between priming, nutrient limitation, and soil moisture availability in arid ecosystems. In a similar incubation study aimed at identifying nutrient limitations in the Mojave Desert, *Larrea tridentata* soils were found to be C limited and that addition of an N source depressed respiration while interspace soils were limited by both C and N (Billings et al. 2004; Schaeffer et al. 2007). Glutamic acid contains both C and N (C:N = 5) and so this amendment increases respiration in interspace soils equally across CO$_2$ treatments leading to consistently positive priming throughout the incubation. The initial positive priming seen in *L. tridentata* soils indicates an initial alleviation of C limitation that results in the highest priming rate, presumably through co-metabolism of glutamic acid and existing SOC. This initial peak is then followed by a decrease in respiration indicative of C immobilization eventually leading to negative priming (Fig 4.5). Over a longer period of time (>24 hours), addition of glutamic acid leads to further C limitation once some amount of available substrate is mineralized. This ultimately decreases SOC mineralization relative to samples that did not receive glutamic acid. We predict that adding a simple C amendment that does not contain N would eliminate the negative priming by increasing the C:N of available substrate. This result highlights that the C:N of C flux can impact mineralization rates and may be a control on how eCO$_2$ affects existing SOC stocks.

The main response to eCO$_2$ in this experiment was in soils amended with water rather than glutamic acid, pointing to a change in the existing microbially available pool of C in these soils. A major control on microbial respiration is DOC concentration as both microbial access to and uptake of C requires solubilization (Schimel and Schaeffer 2012). This pool is particularly critical in arid soils that are characterized by pulsed mineralization during and shortly after precipitations events where microbes are limited by access to substrate. We explored the effect of DOC concentration on respiration by using the natural variation in DOC between cover types
and CO$_2$ treatments. We found that DOC is strongly related to respiration rate in ambient CO$_2$ soils regardless of if the soils are amended with water or glutamic acid and that this relationship is consistent at 4 hours all the way to 168 hours (Fig 4.9). In eCO$_2$ soils though, DOC is only correlated with respiration in water amended soils in at 4 hours and not at all in soils amended with glutamic acid. This trend is driven in part by the slightly lower concentration of DOC in *L. tridentata* soils exposed to eCO$_2$ (Fig 4.2). Despite the strong relationship between DOC and respiration, there is no correlation in DOC and cumulative primed C at 4 or 168 hours (Table 4.3). This may be related to the quality (C:N) of DOC rather than just the absolute quantity, and how added glutamic acid impacts C:N of the pool of available substrate. We were unable to measure N in our DOC extractions but future studies should attempt to quantify C:N or manipulate C:N in order to understand this potential control on priming.

**CONCLUSION**

This experiment demonstrates how soil mineralization and priming are affected the eCO$_2$ effects on microbial substrate availability. While in general respiration rates are controlled by the amount of substrate available, priming may be more sensitive to the C:N of this pool and any added simple C substrate. Under eCO$_2$ we would expect higher C:N in belowground C flux due to increased C fixation. This higher C:N substrate would lead to higher rates of priming and loss of SOC. Soils under perennial shrub cover are less susceptible to overall mineralization relative to interspace soils, however and long-term exposure to eCO$_2$ may therefore result in decreasing priming rates over time as higher microbial activity results in less available forms of SOC.

The respiration responses we identified in this short-term incubation highlight the complex interaction between substrate limitation and soil moisture availability. Changes in the frequency and intensity of precipitation events driven by climate change will undoubtably have a
strong effect on the soil microbial response to eCO2. Furthermore, we show that interspace soils are susceptible to high rates of priming and changes in desert vegetation cover structures, such as encroachment of invasive grasses in the Mojave Desert, may cause increased C flux to these soils and further loss of SOC stocks. Studies on multiple, interacting effects of anthropogenic disturbances are necessary to understand changes in arid ecosystem soil C cycling under future global change.
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