

UC Davis

UC Davis Electronic Theses and Dissertations

Title

Plant Traits, Diversity Metrics, and Environmental Factors Shape Soil Carbon Dynamics in a Restored Grassland

Permalink

<https://escholarship.org/uc/item/0wr7q62x>

Author

Sebastian, Laurel Taylor

Publication Date

2023

Peer reviewed|Thesis/dissertation

Plant Traits, Diversity Metrics, and Environmental Factors Shape Soil Carbon Dynamics in a
Restored Grassland

By

LAUREL TAYLOR SEBASTIAN
THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

Ecology

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

Approved:

Jennifer Funk, Chair

Valerie Eviner

Cristina Lazcano

Committee in Charge

2023

Laurel Taylor Sebastian, 997594975

Plant Traits, Diversity Metrics, and Environmental Factors Shape Soil Carbon Dynamics in a
Restored Grassland

Abstract

In the era of unprecedented biodiversity loss and a subsequent loss of ecosystem function, a core goal of restoration ecology is to identify planting strategies to restore ecosystem functions like soil carbon sequestration. Plant diversity, functional groups and functional traits can all be leveraged to improve ecosystem function, but there are major gaps in our understanding of which strategies are most impactful for increasing soil carbon (C). In this study, we seeded common California grassland species in 210 experimental plots and examined how plant functional groups, functional traits and diversity metrics influenced five unique soil carbon pools. We also evaluated effects of precipitation and land use history treatments on soil C pools across two years to examine how environmental conditions mediate plant-soil interactions, and if there are time lags in those effects. Land use history was the biggest single predictor of soil C, with the duration of shrubs on the site prior to our experiment outweighing the effects of all other variables. In linear mixed-effects model comparisons, environmental variables (precipitation, land use and year) alone best explained particulate organic carbon (POC), while adding species diversity metrics improved predictions for total carbon (TC), microbial biomass carbon (MBC) and mineral-associated organic carbon (MAOC). However, functional and species diversity correlated negatively with soil C pools in some environmental conditions, showing that diversity itself may not always boost soil C levels. Water-extractable organic carbon (WEOC) did not vary with any plant or environmental model. Analyzing effects of individual variables, we found that diversity, functional trait, and functional group correlations varied across soil C pools,

environmental context, and the year of vegetation measured. These results suggest that different plant strategies contribute to soil C pools through disparate pathways on various timescales. Our study highlights how dynamic soil C is in grassland systems and that additional work is needed before restoration managers can use trait-based approaches to sequester soil C in various site contexts.

Introduction

Restoration ecology seeks to maintain or improve core ecosystem functions by restoring biotic and abiotic conditions (Palmer et al. 1997). Two main approaches in plant-based restoration aim to restore function: reintroduce a diverse mix of plant species, or introduce plant species or functional groups known to improve a given ecosystem function (Cardinale et al. 2012, Carlucci et al. 2020). In recent decades, ecologists have also employed functional trait approaches to guide restoration, with the assumption that traits (measurable characteristics like rooting depth) provide a mechanistic understanding of how plants affect their environment (Funk et al. 2008, Laughlin 2014). However, many gaps remain in our knowledge of which functional groups and traits most impact ecosystem functions, and how they compare to biodiversity-based approaches (Poirier et al. 2018, Carlucci et al. 2020). As climate and land use changes progress, we have the added challenge of identifying restoration strategies that succeed across different environmental conditions (Harris et al. 2006). Continued research on the roles of traits, functional groups, and diversity on ecosystem functions will bring us closer to deploying more effective planting strategies to meet restoration targets.

A restoration goal of great interest is building soil carbon (C) to help mitigate climate change and regulate other critical ecosystem services including soil stability, nutrient availability, and water holding capacity (Follett and Reed 2010, Booker et al. 2013). Soil C may also increase the financial stability of working landscapes through its sale on emerging carbon markets (Follett and Reed 2010, Eastburn et al. 2017). However, soil C is a highly dynamic carbon pool composed of unique soil carbon components, and much is still unknown about how plant functional traits, functional groups, and environmental conditions interact to alter soil C in short and long term carbon pools (Butenschoen et al. 2011, Conant et al. 2017, Poirier et al.

2018, Carlucci et al. 2020). Knowledge gaps in soil C formation and loss are crucial to address in grasslands since grasslands represent one of the largest carbon pools (50 tons C/ha; Dondini et al. 2023), store most of their carbon long-term underground (Paruelo et al. 2010), and are imperiled globally (Bai et al. 2008). In particular, California grasslands need further research since they have different biotic and abiotic conditions than commonly-studied mesic grasslands (Booker et al. 2013), but cover over 10% of the state (Corbin et al. 2007) and may offer a more stable carbon sink than forests under climate change (Dass et al. 2018).

The five soil C components included in this study stem from diverse plant-soil interactions and vary in their long-term stability and contribution to C sequestration (Poirier et al. 2018, Dynarski et al. 2020). Total carbon (TC) is the most commonly measured form of soil C and encompasses all the pools described below. TC is therefore not as closely linked to any one plant-soil interaction or timeline of persistence in the soil, but it can be tracked over time to determine if an ecosystem is acting as a net carbon source or sink (Conant et al. 2011).

Particulate organic carbon (POC) consists of fragments of plant matter that are vulnerable to decomposition, but feed the soil C cycle and can persist for 1-50 years (Lavalley et al. 2020, Cotrufo and Lavalley 2022). Water extractable organic carbon (WEOC) represents a relatively small but active pool of bioavailable carbon derived primarily from root exudates and labile tissues, and is important in stimulating microbial growth (Bolan et al. 2011, Haney et al. 2012, 2018). Microbial biomass carbon (MBC) reflects the live soil microbial community. While microbes themselves are short lived, microbial processing of plant C into chemically and physically stable forms is the primary mechanism for long term C sequestration in many systems (Cotrufo and Lavalley 2022). Mineral-associated organic carbon (MAOC) is the pool of carbon derived from that microbial processing as well as direct adsorption of C molecules onto minerals

in the soil, and is thought to remain in the soil for 10-1000 years (Cotrufo et al. 2019). However, MAOC can reach a saturation point depending on the mineral surface area of the soil type, which can limit expected plant contributions to this pool (Cotrufo and Lavelle 2022). The unique characteristics of each pool are key in understanding how management influences soil C and how, together, they determine long-term soil C sequestration and climate change mitigation (Dynarski et al. 2020).

Recent studies have attempted to find mechanistic links between soil carbon and various plant functional trait measures, with increasing focus on root traits as the drivers of soil C formation and loss (see Table 1). Based on the mass-ratio hypothesis, community weighted mean (CWM) trait values help identify when dominant species or traits in a community drive a desired function (Grime 1998, Wang et al. 2021). Some researchers have found that trait values of slow-growing, resource conservative plants (e.g. high leaf mass per area (LMA), high root tissue density (RTD), low photosynthetic rate (Asat)) are linked to recalcitrant litter that decomposes slowly and builds up as POC (Aerts and Chapin 2000, De Deyn et al. 2008). Opposite trait values associated with a more resource acquisitive strategy and labile plant tissues may increase soil C by stimulating microbial growth and processing of carbon into more stable pools like MBC or MAOC (Poirier et al. 2018, Lavelle et al. 2020, Hanisch et al. 2020). Therefore, part of the challenge in pinpointing traits that broadly boost soil C arises because various trait values can specifically enhance different soil C pools (Poirier et al. 2018). Beyond individual traits, the total functional niche space occupied by a community (functional richness, FRic) and the evenness of that trait distribution (functional evenness, FEve) can improve ecosystem functions (Díaz et al. 2007, Wang et al. 2021). Functional trait diversity can increase soil C via increased plant biomass due to more efficient resource use (niche complementarity), as well as enhanced

resources for microbes through a diversity of root and litter types (Chen et al. 2020). Careful examination of various functional trait measures and particular carbon pool components can help tease apart which mechanisms are driving soil C change (Billings et al. 2021).

In other cases, plant species diversity or the presence of specific functional groups can also play a positive role in building soil C (Díaz et al. 2007, Chen et al. 2020). Similar to functional diversity, higher species diversity tends to increase soil C sequestration rates and stocks via increased plant productivity and litter inputs (Fornara and Tilman 2008). However, it can also cause C loss by additional fresh C inputs stimulating microbes to accelerate decomposition of more stable C (i.e. priming effects) (Chen et al. 2020). While a global review showed a net positive effect of species diversity on soil C, results are not consistent across all environmental conditions and there is a lack of data on Mediterranean grasslands (Chen et al. 2020, 2022). Similar to a trait-based approach, targeting functional groups (e.g. forbs vs. grasses) can offer a simple and mechanistic strategy to restore ecosystem function (Reich et al. 2004, Fornara and Tilman 2008). For example, perennial grasses have been shown to aid in carbon sequestration through their deep and abundant root biomass and conservative resource use strategy (Koteen et al. 2011, Anacker et al. 2021). Annual forbs and grasses are studied less frequently, but are more likely to have an acquisitive, drought-escape strategy in California in which less biomass is allocated belowground (Welles and Funk 2021). More research is needed on whether such acquisitive annuals lead to a net C loss via priming or an efficient conversion of C into the MAOC pool (Dynarski et al. 2020, Wen et al. 2021). Many grassland studies testing trait or species effects on soil C are conducted in perennial-dominated grasslands (Fornara and Tilman 2008), and rely on measurements of current vegetation at the time of soil collection (Zuo et al. 2016). In annual grassland systems, however, the plant composition can vary significantly

between years (Young et al. 2005), which could have subsequent effects on the buildup of soil C. Moreover, carbon inputs from plants move through different soil C pools on various timescales (Fossum et al. 2022), so it may be important to consider the effects of both the current and prior year plant composition on soil C pools in annual-dominated grassland systems.

While examining plant effects on soil C, it is also crucial to test how these relationships shift across environmental conditions including precipitation and land use history. Some authors caution that strategies to increase soil C in mesic grasslands may not hold up in arid or semiarid grasslands where primary productivity can be low (Booker et al. 2013). Climate change induced drought may turn grassland carbon sinks into sources by stimulating microbial stress and respiration (Hoover and Rogers 2016), but others find consistent resilience to drought (Zhang and Xi 2021). Under the stress gradient hypothesis, one recent study suggests that plant diversity may aid ecosystem function most under stressful conditions, meaning a diversity-centered strategy would be even more important under drought (Maestre et al. 2009, Chen et al. 2022). In the restoration context, land managers also contend with prior land use histories of a site influencing baseline soil carbon stocks and soil structure. For example, shrub encroachment is common in grasslands globally and can increase soil carbon while cultivation usually depletes soil carbon stocks (Zavaleta and Kettley 2006, Conant et al. 2017, Luong 2022). Such land use history can change plant and microbial access to soil organic matter and shift the subsequent sequestration and cycling of soil carbon (England et al. 2016, Haddix et al. 2020, Wang et al. 2021). With the increased variation in annual precipitation (Berg and Hall 2015) and the extreme variation in land use histories, it is more important than ever to test how plant-soil interactions change across environmental conditions (Butenschoen et al. 2011).

In this study, we explore how plant diversity, traits and functional groups correlate with five unique soil C pools under different environmental conditions. We leveraged a restoration experiment that seeded 32 common grassland species in various combinations across 210 plots subjected to either 50% or 125% of average annual precipitation and two land use history conditions (two or six years of prior shrub occupation). We then tested whether models built from functional groups, trait, or diversity metrics best predict five pools of soil C, and which variables were significantly correlated with each of the soil C pools. We also evaluated whether the current or prior year plants drive those correlations, and how the plant effects are changed by environmental conditions. We hypothesized that soil C pools would correlate positively to plant diversity but would vary in their relationships to certain traits and functional groups and in the timelines of their responses to plant inputs. We also expected more precipitation and longer shrub land use to increase soil C levels on average, and to alter the relationships of plant variables with soil C pools.

Materials & Methods

Experimental design

We established 210 experimental plots in 2019 at the UC South Coast Research and Extension Center in Irvine, CA (33°41'N, 117°43'W) in a Mediterranean climate (330mm mean annual precipitation, 16.9°C mean annual temperature). The site was established on San Emigdio sandy loam soils with a flat slope, moderate alkalinity (pH ~8.2), and <5% calcium carbonate (USDA n.d.). Bulk density at the site averaged 1.20g cm⁻³ at 0-15cm (standard deviation = 0.05, n=30). The experiment followed a factorial block design with the 210 grassland plots distributed

across 15 precipitation shelters that experienced one of two precipitation treatments and one of two land use histories (see treatment sample sizes and plot layout in Appendix A).

To assess how precipitation alters plant-soil interactions, we conducted the experiment in a $30 \times 30\text{m}$ area in 15 rainout shelters ($3\text{m (w)} \times 7.3\text{m (l)} \times 2.6\text{m (h)}$) containing 14 0.56 m^2 plots each. Eight shelters received 50% of average January-April rainfall (110mm) and seven shelter received 125% (275mm) of average January-April rainfall. These treatments represent typical low and high rainfall amounts during dry and wet years, respectively. We controlled precipitation by deploying transparent tarps above all shelters during major rainfall events ($>0.25\text{mm}$) between January and April, and then irrigating plots (two to three times per week) to reach target values (emitter height 1.5 m; flow rate 14 mm/h; $>75\%$ distribution uniformity). If tarps were not deployed in time for rain, irrigation was adjusted to reach target values. However, with historic rains in 2023, 50% plots appeared to absorb additional water from surrounding soils. Soil moisture sensors (EC-5 sensor, Decagon Devices, Pullman, Washington) installed 15 cm below the soil surface in each shelter tracked actual soil moisture readings (see Appendix A, Figure A4). Tarps were removed entirely between May and December each year, although rainfall during the summer month was minimal. During the October-May growing seasons, local weather varied from 177mm rainfall and 17.0°C in 2021/2022 to 466mm rainfall and 17.6°C in 2022/2023.

The precipitation shelters were originally constructed and planted in two phases for previous studies on short-lived shrubs (Funk et al. 2021, Atamian and Funk 2023), which resulted in two distinct land use histories before the start of our study. Native shrubs were grown for six years (2013-2019) in nine shelters, and two years (2017-2019) in six shelters (see Appendix A for more details on prior studies). Prior to shelter construction and shrub planting,

the areas were disced yearly and planted with barley, so the shelters with six years of shrub land use history were also removed from tilling four years earlier. During the summer of 2019, we cleared shrubs from all plots, tilled and raked the soil, and removed much of the large taproots from the top 15cm of soil, but deeper roots and shallow fine roots likely remained. Due to the significantly higher soil C values registered in our plots with the six years of shrub land use history (Appendix A, Figure A5), we included “land use” as a treatment with two levels (two years of shrubs and six years of shrubs) throughout our study (Figure 1). These two land use conditions mirror restoration scenarios in which the prior site history (e.g., shrub-encroached grassland vs. tilled cropland) may result in different soil C conditions, which may moderate subsequent plants and plant effects on soil C. Although the previous studies also had precipitation treatments, the precipitation treatments varied in their levels and locations between each study and did not seem to influence soil C pools (data not shown). Any lingering precipitation treatment effects from prior studies are controlled for by including the precipitation shelter as a random effect.

We seeded each of the plots in fall of 2019 with 10 native California grassland species out of a 32 species pool (Appendix A). The 10 native species were originally seeded at unique ratios modeled to meet four target functional trait ranges as explained in Laughlin et al. (2018). However, we found no differences in community composition and trait averages between those four groups after the initial seeding and they did not significantly correlate with any soil C pool (data not shown). Therefore, the trait groups are not examined in this work and will be published separately. Rather, this study examines the actual species composition and trait values achieved. In December 2020, we also seeded half of the plots with an invasive grass (*Festuca perennis*) to assess the effects of a common invader. Due to low initial recruitment, we repeated the invasive

seeding treatment in 2021 and allowed two other invasive grasses (*Bromus diandrus* and *Bromus madritensis*) to recruit naturally in the same plots. We weeded all other species out of plots throughout the growing season. The presence of invasive species did not have a direct effect on any soil C pool, so these species are analyzed in the same way as all others in the study. Eight *Festuca perennis* monoculture plots were excluded in our final analyses, leaving 202 total mixed-species plots in our analyses.

A single observer estimated percent cover of each of the 35 species (32 natives and 3 invasives) each spring from 2021 to 2023 (as in Funk et al. 2015). For the 31 species (29 natives and 3 invasives) that remained in the experiment in 2022 and 2023, percent cover estimates were summed within functional groups: perennial grasses (n=8), annual grasses (n=5), perennial forbs (n=5) and annual forbs (n=13) (see Appendix A, Table A1 for species). To explore the relationship between plant functional traits and soil carbon pools, we obtained average trait values for each species from prior studies (Appendix C) (Funk et al., in review). We included ten traits (Table 1) that are commonly measured, are theorized to affect soil carbon or other major ecosystem functions in grasslands (Hanisch et al. 2020), and were available for all 31 species.

Table 1: Functional traits included in this study and their expected effect, increase (+) or decrease (-), on soil carbon in grasslands. *These traits were included in the multi-trait model used for model comparisons due to their lack of collinearity (correlation factors < 0.5).

Functional Trait	Predicted Effect	Predicted Mechanism of Impact on Soil Carbon	Sources
Leaf mass per area (LMA, g m ⁻²) *	+	Dense leaves of resource-conservative plants create more recalcitrant litter and buildup of POC.	(De Deyn et al. 2008, Zuo et al. 2016)
Leaf nitrogen content (LNC, g N kg ⁻¹)	+ / -	LNC facilitates photosynthesis; high LNC plants are generally fast-growing and release more exudates underground which can stimulate MBC. MBC is essential for MAOC formation but can also drive priming (decomposition/ release of CO ₂).	(De Deyn et al. 2008, De Vries et al. 2016, Chen and Chen 2018, Hanisch et al. 2020, Wen et al. 2021)
Light saturated photosynthetic rate (Asat, μmol CO ₂ m ⁻² /sec)	+ / -	Correlated with LNC and similar impacts as above. Low Asat plants are also more drought tolerant, possibly allowing for more contribution to soil C in drought conditions.	(See above and Nguyen et al. 2016)
Water use efficiency (WUE, g C kg H ₂ O ⁻¹)	?	High WUE plants sequester more carbon per unit of water and can maintain growth in dry conditions, which may be important for soil C effects in drought conditions. (No studies on soil C and WUE found).	(Ge et al. 2019)
Root tissue density (RTD, g cm ⁻³) *	+ / -	Dense root litter of resource-conservative species is more recalcitrant and promotes POC, but reduces root exudation that stimulates WEOC/MBC/MAOC.	(Craine et al. 2005, De Deyn et al. 2008, De Vries et al. 2016)
Root length density (RLD, m root cm ⁻³ soil) *	+	Abundant roots of resource-acquisitive species provide habitat for microbes (MBC); root hairs help form microaggregates, which stabilizes POC/MAOC.	(Gould et al. 2016, Poirier et al. 2018)
Root mass fraction (RMF, g root g ⁻¹ plant) *	+	Resource-conservative species allocate more biomass underground, and root litter is generally more recalcitrant than shoots, building POC and providing more root surfaces for MBC.	(Craine et al. 2005, De Deyn et al. 2008)
Root diameter (Rdiam, mm) *	+ / -	Thick roots build POC through recalcitrant litter, but thin roots can increase soil aggregation and C stabilization through MBC. Weigelt et al. (2021) also place Rdiam/SRL on a separate “collaboration gradient” to describe mycorrhizal associations, but that has not been clearly linked to soil C in grasslands.	(Poirier et al. 2018, Hanisch et al. 2020, Weigelt et al. 2021)
Specific root length (SRL, cm root g ⁻¹ dry root)	+ / -	Thin roots of resource-acquisitive species are dispersed throughout the soil and exude more carbon, stimulating MBC to decompose or stabilize C.	(De Vries et al. 2016, Poirier et al. 2018, Wen et al. 2021)
Seed mass, (SM, g)	?	No known correlation to C, but SM has been associated with drought-survival in seedlings and community biomass.	(Roscher et al. 2013, Harrison and LaForgia 2019, Hanisch et al. 2020)
Functional richness (FRic) and Functional evenness (FEve)	+	FRic indicates the amount of multidimensional niche space of the plant community. FEve is the evenness of the abundance distribution in the filled niche space. Both relate to trait diversity and niche complementarity and have been found to enhance soil C.	(Díaz et al. 2007, Butterfield and Suding 2013, Wang et al. 2021)

Soil collection & analysis

We collected soils during the third (2022) and fourth (2023) years of this project during peak growth (March-April). We cleared litter from the soil surface before collecting 2cm diameter soil cores (two in 2022 and three in 2023) at 0-15cm from each plot. Probe locations were randomly selected from a 36-cell grid within the plot at least 10cm from the plot edge while avoiding destruction of plant crowns and prior probe holes.

We determined the concentration of total carbon (TC) via elemental combustion analysis (Costech 4010, Pioltello, Italy) on 15mg of sieved (2mm) and oven dried (60°C) soil. We extracted WEOC within three weeks of field collection and froze extracts at -20°C for less than 3 months before analysis (Haney et al. 2012, 2018). For WEOC analysis, we combined 4g fresh soil with 40ml distilled water, shook for 10 minutes on a mechanical shaker at 210rpm, and centrifuged for 10 minutes at 3500rpm before vacuum filtering the supernatant through 0.45µm Cytiva Whatman mixed cellulose ester membrane filters (Rees and Parker 2005, Haney et al. 2012). Within six weeks of soil sample collection, we analyzed MBC using the chloroform fumigation extraction method (Horwath and Paul 1994). Briefly, 6g of field-moist soil were fumigated with chloroform for 24 hours before extraction with 20ml of 0.5M K₂SO₄. In parallel, we extracted a set of non-fumigated samples. Extracts of fumigated and non-fumigated samples were filtered through Fisherbrand Q5 filter papers, and frozen at -20°C until analysis within nine months. We acidified WEOC and MBC extracts to pH 2 using sulfuric acid (H₂SO₄) before analysis on a TeleDyne-Tekmar Fusion total organic carbon analyzer (Mason, Ohio) at the University of Wyoming (Rees and Parker 2005, Rhymes et al. 2021). We calculated MBC as the difference between the amount extracted from the fumigated and unfumigated soil, divided by a 0.35 extraction correction factor (Voroney et al. 2007). We determined POC and MAOC by size-

fractionation (> or <53 μ m, respectively) using a Fritsch Analyzette 3 (Pittsboro, NC) after shaking 10g of air-dried soil, five glass beads and 30ml 0.5% sodium hexametaphosphate for 18 hours at 210rpm (Cotrufo et al. 2019). We then dried both fractions at 60°C and ran 30mg of hand-ground samples through a combustion analyzer (as above). Sample sizes for each soil C pool varied due to financial and time constraints. After monoculture plots (n=8) and outliers were removed, we had the following sample sizes for each soil C pool in each year: TC (197 in 2022, 199 in 2023), MBC (49 in 2022, 189 in 2023), POC (89 in 2022, 192 in 2023), MAOC (96 in 2022, 198 in 2023), and WEOC (0 in 2022, 196 in 2023).

Statistical analysis

We performed statistical analyses in R (<https://www.r-project.org/>) using linear mixed-effects regression (LMER) models. We log-transformed TC in all analyses to fit assumptions of normality. We examined and removed outliers beyond two standard deviations in all soil C pools. However, we retained WEOC measures below two standard deviations because the low values were all from the same precipitation shelter and were therefore more likely due to site variation than measurement error. Because functional trait values were more variable, we removed trait values beyond three standard deviations. We also log transformed RLD and SM trait values to fit assumptions of normality before we scaled all traits. Functional groups and diversity metrics were also scaled around zero to aid in comparing effect sizes. In all models except WEOC, we included year, precipitation and land use as fixed effects, and the precipitation shelter (hereafter ‘shelter’) as a random effect. Precipitation and land use were included as interaction terms to see how they moderated other variables, but year was only included as a

main effect since it is included in models mostly to control for the repeated measure of each plot. WEOC was only measured in 2023, so we did not include year in any WEOC models.

Our first round of analyses set out to test whether functional traits, functional groups, functional trait diversity, or species diversity metrics improved the prediction of each soil C pool over models that only contained the environmental variables (year, precip, and land use). We also built a null model ($C \text{ pool} \sim 1 + (1|Shelter)$) for each soil C pool and evaluated models using AIC (Akaike information criterion) values (Table 2) (Akaike 1998). The set of four LMER model groups was replicated twice: once with all variables calculated from the plant community the same year the soil was collected (“current year”), and once with all variables calculated from the prior year plant community (“prior year”). This means that (i) 2023 soil C values were compared to 2023 and 2022 plant species and traits, and (ii) 2022 soils were compared to 2022 and 2021 plant species and traits. The functional traits values used are community weighted mean (CWM) values calculated as the average trait value in the plot, weighed by the species cover percentages (Conti and Díaz 2013). We narrowed traits for the multi-trait model from ten traits to five traits that span above and belowground measures by removing the most collinear traits until all traits had correlation factors < 0.5 (included traits: LMA, RTD, RLD, RMF, R_{diam}). Functional groups were calculated by summing the percent cover of all species in each group per plot. Legumes were tested in preliminary functional group models, but they were mostly insignificant (except a positive correlation with MBC; data not shown), so we removed legumes to avoid double counting those species in the annual forb group. The functional diversity model included both functional richness (FRic) and functional evenness (FEve) (calculated using the ‘FD’ package in R), while the species diversity model included species richness and evenness. To compare AIC values between models with the same sample size for each soil C pool, we

removed all rows with NA values (from outlier removal) in any of the predictor variables above (samples sizes are reported in Table 2).

Our second set of analyses examined soil C correlations with individual predictor variables, since we could not run collinear traits in the same model and individual correlations can be obscured in multi-variate models. We ran LMER models for each soil C pool against percent cover for each of the four functional groups, CWMs for the 10 functional traits, species richness, species evenness, FRic, and FEve. Each of those models retained precipitation, land use and year as main effects, structure as a random effect, and interaction terms with precipitation and land use. These regressions were also analyzed in two groups: once for the current year value and once for the prior year value. The models were run on the full dataset available for each soil C pool, and only significant model terms (p -value < 0.05) are reported below (Table 3; Figures 1-5). However, sample sizes for significant models varied by 1-10 rows based on the number of NA values for each variable, so the dataset was reduced until each significant model in Table 3 had the same sample size for each soil C pool. This allowed us to compare R^2 and AIC values within each C pool. Any terms that were no longer significant (<0.05) in the slightly smaller dataset were not included in results below. All reported R^2 values are marginal.

Results

Environmental treatment effects on soil C pools and vegetation

The environmental treatment variables included in all models (year, precipitation, and land use) were significant predictors of all soil C pools except WEOC (Figure 1, Table 3). Land use was the strongest single predictor for all soil C measures except WEOC; plots with six years of prior shrub history had higher soil C values for TC ($p < 0.001$, $F = 31.43$), MBC ($p = 0.022$,

F=6.88), POC ($p=0.001$, $F=33.32$), and MAOC ($p<0.001$, $F=30.85$). Year was significant for MBC ($p<0.001$, $F=47.17$) and POC ($p=0.022$, $F=5.31$), with both measures showing a greater C value in 2023. Precipitation only had a significant effect on MAOC ($p=0.021$, $F=6.97$), with 125% precipitation plots having higher MAOC values. Precipitation and land use also interacted significantly with other explanatory variables and those results are reported below (Figures 2-5).

Vegetation also varies with these three environmental variables. Graphs of the percent cover of each functional group by year, precipitation treatment and land use history are shown in Appendix A, Figure A3. Briefly, total cover and annual grass and forb cover was higher in 2023 while perennial grass cover was higher in 2022. All cover categories were higher in 125% precipitation plots except annual forb cover was higher in drought plots. There was no difference in plant cover between land use types except a higher percent cover of perennial grasses in plots with only two years of shrub land use history.

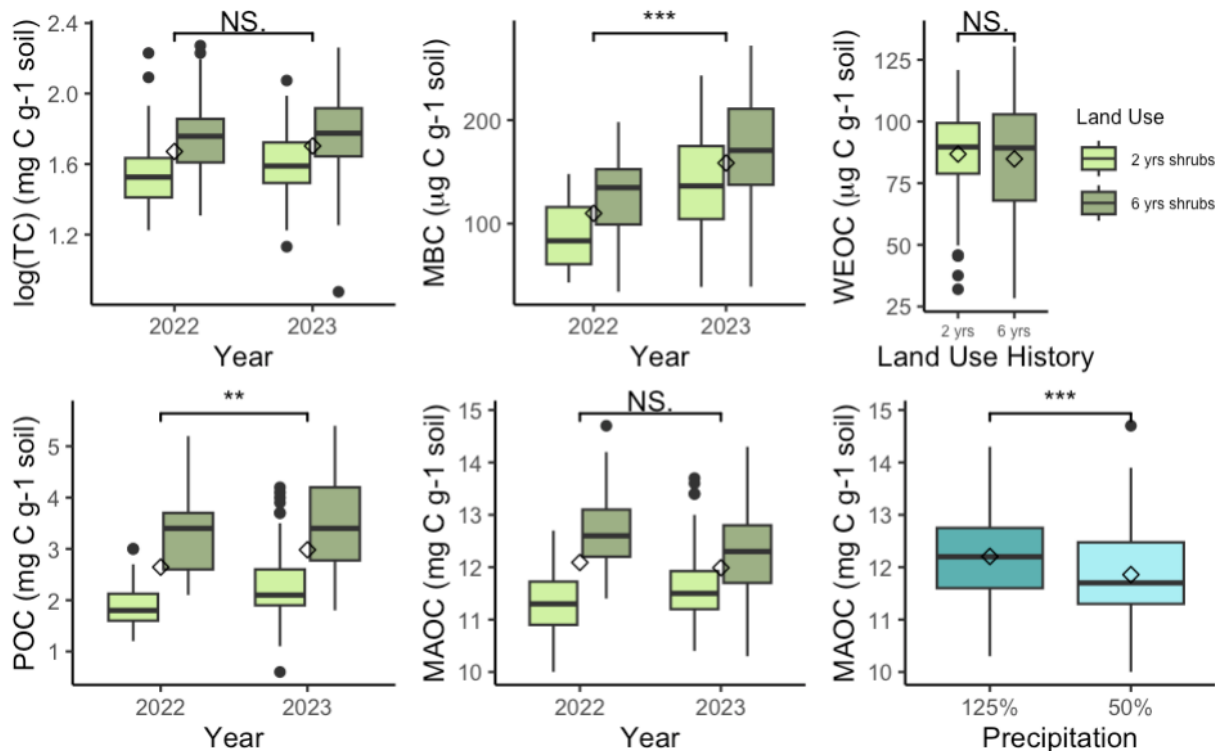


Figure 1: All effects of year and land use history, and the one significant effect of precipitation on soil C pools. Every soil C pool except water extractable organic carbon (WEOC) was higher in the plots with six years of shrub

land use history. Microbial biomass carbon (MBC) and particulate organic carbon (POC) were significantly higher in 2023 than 2022. Only mineral-associated organic carbon (MAOC) was significantly higher in wetter (125% precipitation) plots. Diamonds show means for each year, land use history, or precipitation treatment, depending on graph. See Table 3 for significance statistics. Stars indicate significance levels of p-values from simple T-tests and vary slightly from multi-variate p-values reported in Table 3. * p<0.05, ** p<0.01, ***p<0.001

Model comparisons within each soil C pool

For each of the five soil C pools, we tested whether null, environmental, functional group, functional trait or diversity models performed best by comparing AIC values (Table 2). Environmental models including year, precipitation and land use had significantly lower AIC values than null models for each soil C measure other than WEOC. For WEOC, the lowest AIC value is associated with the null model. Prior year species diversity models best predicted TC and MBC, although the prior year functional group model performed nearly as well for MBC. MAOC was best predicted by current year species diversity, although the AIC value was only slightly lower than the environmental model. Trait-based models were also significantly different than environmental models for MBC and MAOC, but the higher AIC values indicated that the slight predictive benefit of including functional traits does not outweigh the cost of additional model complexity. POC was best predicted by the environmental variables alone, meaning no other additional variables improved the model. Significant model terms and R² values and for each model and are included in Appendix B.

Table 2: Model AIC comparisons for each soil carbon pool. The environmental model is built with only precipitation, land use and year, and each model line below that adds in the elements listed in the model variables column. Lowest AIC values are bolded. P-values in the environmental variables row compare the environmental model to the null model in an ANOVA test. All other p-values are from ANOVA comparisons between the model in that row and the environmental model, so they represent whether there is any significant difference in model prediction by adding the variables in that row. The five traits are leaf mass area, root tissue density, root length density, root mass fraction, and root density. The functional groups are perennial forbs, annual forbs, perennial grasses, and annual grasses. FEve is functional evenness and FRic is functional richness. Vegetation year refers to whether the variables in that row are calculated with current or prior year plant cover data. Additional model details are available in Appendix B.

Model Type	Model Variables	Vegetation Year	Model AIC and P Values				
			TC (n=333)	MBC (n=201)	POC (n=236)	MAOC (n=246)	WEOC (n=171)
Null model	1 + (1 Shelter)	Both	-123.90	2141.1	553.43	547.61	1469.7
Environmental Variables (included in all models)	Precip + Year + Land Use + (1 Shelter)	Both	-139.50 (p<0.001) ***	2104.0 (p<0.001) ***	532.97 (p<0.001) ***	528.71 (p<0.001) ***	1473.5 (p=0.911)
Functional Traits (CWM)	+ (5 Traits) * (Precip + Land Use)	Current	-123.16 (p=0.682)	2121.2 (p=0.617)	548.13 (p=0.462)	531.68 (p=0.028) *	1492.5 (p=0.749)
	+ (5 Traits) * (Precip + Land Use)	Prior	-118.86 (p=0.858)	2108.9 (p=0.049) *	553.56 (p=0.854)	548.33 (p=0.795)	1493.9 (p=0.844)
Functional Groups (% Cover)	+ (4 Func. Groups) * (Precip + Land Use)	Current	-126.63 (p=0.469)	2113.4 (p=0.265)	537.89 (p=0.839)	538.30 (p=0.217)	1482.0 (p=0.278)
	+ (4 Func. Groups) * (Precip + Land Use)	Prior	-121.87 (p=0.968)	2102.3 (p=0.012) *	549.13 (p=0.087)	537.24 (p=0.276)	1482.5 (p=0.578)
Functional Diversity	+ (FRic + Feve) * (Precip + Land Use)	Current	-135.60 (p=0.231)	2110.8 (p=0.515)	535.11 (p=0.131)	531.14 (p=0.144)	1477.5 (p=0.242)
	+ (FRic + Feve) * (Precip + Land Use)	Prior	-135.15 (p=0.265)	2112.0 (p=0.675)	542.46 (p=0.867)	538.78 (p=0.922)	1482.0 (p=0.748)
Species Diversity	+ (Evenness + Richness) * (Precip + Land Use)	Current	-133.07 (p=0.473)	2108.5 (p=0.276)	537.27 (p=0.0260)	527.63 (p=0.042) *	1482.5 (p=0.807)
	+ (Evenness + Richness) * (Precip + Land Use)	Prior	-141.21 (p=0.033) *	2102.2 (p=0.032) *	542.10 (p=0.824)	533.49 (p=0.301)	1481.9 (p=0.734)

Functional trait and functional diversity effects on each soil C pool

In single-trait linear regression models for each of the ten CWM trait values against each C pool, traits rarely predicted soil C (Table 3). Of the ten traits tested, TC was negatively associated with current CWM values for root mass fraction (RMF) ($p=0.038$, $F=4.35$; Figure 2). Only the light saturated photosynthetic rates (Asat) of prior year plant communities were positively correlated with WEOC ($p=0.031$, $F=4.75$; Figure 2). No traits from current or prior year plant communities significantly predicted MBC, POC or MAOC consistently across environmental conditions, but MBC and MAOC had interactive correlations with environmental treatments and traits. Although the precipitation treatment only directly correlated with MAOC, precipitation and prior year leaf nitrogen content (LNC) did have an interactive correlation with MBC ($p=0.013$, $F=6.27$; Figure 2). MBC showed a more positive correlation with this acquisitive trait under well-watered conditions than drought conditions.

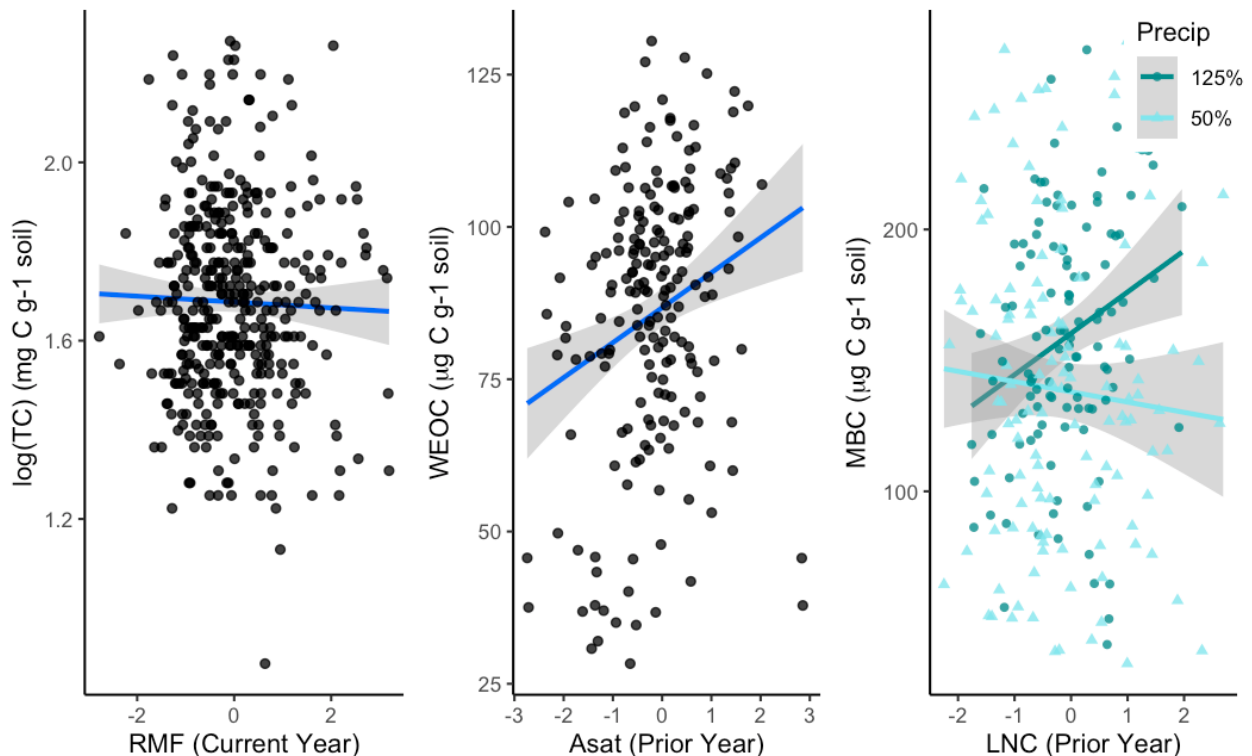


Figure 2: Significant functional trait correlations with total carbon (TC) and water extractable organic carbon (WEOC), as well as a significant trait and precipitation interaction in the microbial biomass carbon (MBC) pool.

Root mass fraction (RMF) of current year plants and TC had a slight negative correlation, while prior year photosynthetic rate (Asat) and WEOC had a positive correlation. Leaf nitrogen content (LNC) had a more positive correlation with microbial biomass carbon (MBC) in 125% precipitation plots. Statistics (P-values, F-values and R²) are listed in Table 3.

The land use history of the plots had three significant interactive correlations with traits in the MAOC and MBC pools (Figure 3). MBC showed a disparate trait response to log transformed root length diameter (RLD) between land use treatments. RLD had a negative correlation with MBC in six year shrub plots, but a positive correlation in two year shrub plots ($p=0.012$, $F=6.45$). In the MAOC pool, land use interacted with current year root diameter (R_{diam}) ($p=0.024$, $F=5.13$) and log transformed seed mass (SM) ($p=0.012$, $F=6.42$). There was little to no trait correlation with MAOC in the relatively carbon rich plots with six years of prior shrub land use history, and a slight negative trait correlation in the two year shrub plots.

While the POC pool did not correlate with any individual functional traits, it did correlate with functional evenness (FEve) and functional richness (FRic) (Figure 3). Current year FEve correlate positively with POC in plots with two years of shrub land use history, and negatively in plots with six years of shrub occupation ($p=0.019$, $F=5.53$). POC also had a positive relationship with current year FRic in drought plots, but a neutral to negative relationship in watered plots ($P=0.019$, $F=5.53$). Overall, functional trait and functional diversity correlations varied substantially between each soil C pool, and correlations were mostly dependent on the environmental context.

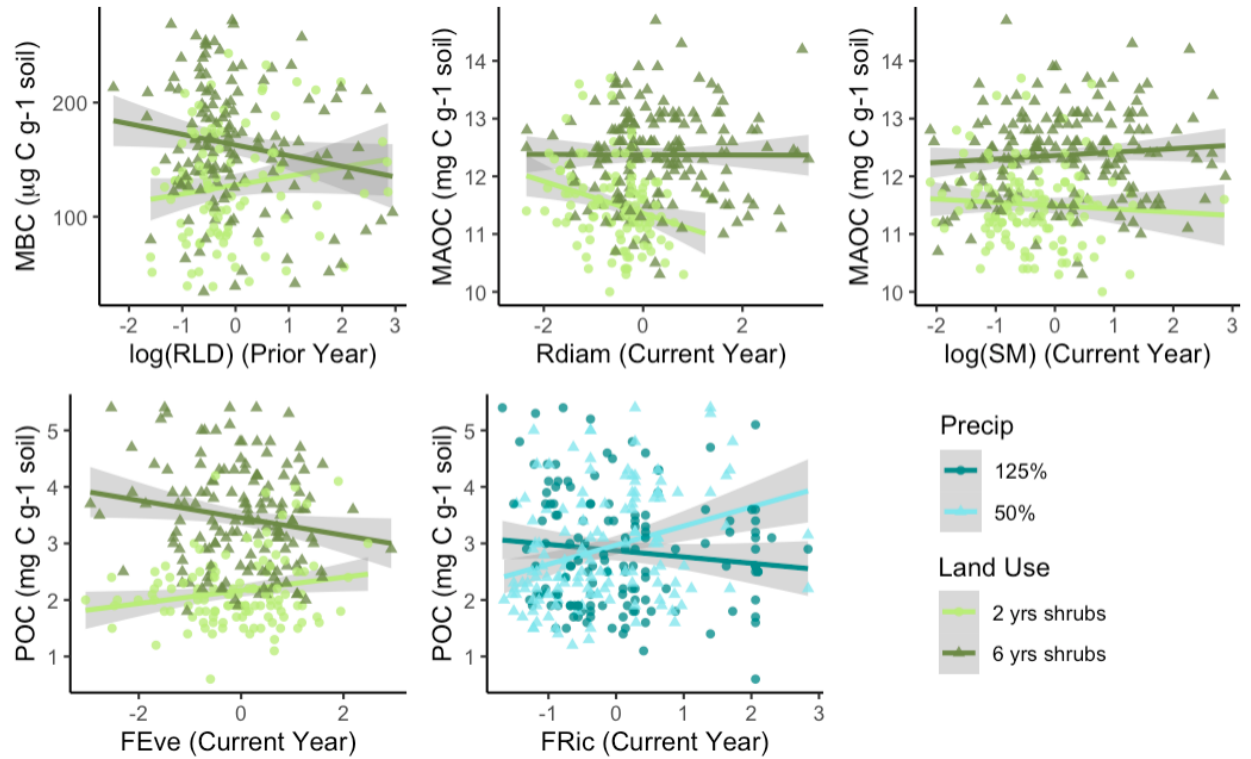


Figure 3: Significant land use and precipitation interactions with community weighted mean trait values, functional evenness (FEve) and functional richness (FRic) for mineral-associated organic carbon (MAOC), microbial biomass carbon (MBC), and particulate organic carbon (POC). Traits are root diameter (Rdiam), and log transformed seed mass (SM) and root length diameter (RLD). Trait and functional diversity correlations were context dependent.

Table 3: Significant correlations between individual variables and soil C pools. Each model related to every line below contained the environmental variables, and interaction terms between the given variable and precipitation and land use treatments. For example, the first line shows that current year RMF (root mass fraction) was significant in the model for that variable ($\log(\text{TC}) \sim \text{Year} + \text{Precipitation} * \text{RMF_C} + \text{Land use} * \text{RMF_C} + (1 | \text{Shelter})$), but environmental interactions with RMF were not significant, so they are not listed in the table. The R^2 and AIC values reflect the entire model for that variable, including interaction terms. Estimates are scaled in that each explanatory variable is scaled around zero.

Soil C Measure	Model Additions	Current/ Prior Year	Scaled Estimate	P-value	F-value	R ²	AIC	Environment al Variables (P-value, F- value)
TC (n=383)	$\log(\text{TC}) \sim \text{Precip} + \text{Year} + \text{Land use} + (1 \text{Shelter})$					0.202	-128.46	Year (0.055, 3.71), Precip (.946, 0.01), Land use (<0.001, 31.43)
Traits	RMF	Current	-0.02	0.038	4.35	0.218	-110.00	
Functional Groups	Annual Grass	Current	-0.02	0.024	5.12	0.216	-110.58	
Diversity	Sp. Richness * Precip	Prior	0.02 (125%), -0.05 (50%)	0.007	7.23	0.222	-113.01	
MBC (n=232)	$\text{MBC} \sim \text{Precip} + \text{Year} + \text{Land use} + (1 \text{Shelter})$					0.261	2403.55	Year (<0.001, 47.17), Precip (0.090, 3.42), Land use (0.023, 6.88)
Traits	LNC * Precip	Prior	8.06 (125%), -8.12 (50%)	0.013	6.27	0.277	2386.81	
	$\log(\text{RLD}) * \text{Land Use}$	Prior	6.01 (6 yrs), 9.78 (2 yrs)	0.012	6.45	0.281	2387.62	
Functional Groups	Perennial Forbs * Precip	Current	7.94 (125%), -9.20 (50%)	0.039	4.29	0.272	2386.96	
	Annual Forbs * Precip	Prior	-10.7 (125%), 12.3 (50%)	< 0.001	14.41	0.296	2377.59	
Diversity	Sp. Evenness * Precip	Prior	-0.38 (125%), -13.69 (50%)	0.028	4.89	0.293	2380	
POC (n=272)	$\log(\text{POC}) \sim \text{Precip} + \text{Year} + \text{Land use} + (1 \text{Shelter})$					0.406	625.27	Year (0.022, 5.31), Precip (0.935, 0.01), Land use (0.001, 33.32)
Traits	None	-	-	-	-	-	-	
Functional Groups	Perennial Grasses * Precip	Current	-0.06 (125%), 0.21 (50%)	0.030	4.75	0.417	635.71	
Diversity	Func. Evenness * Land Use	Current	-0.07 (6 yrs), 0.13 (2 yrs)	0.019	5.53	0.416	635.95	
	Func. Richness * Precip	Current	-0.16 (125%), 0.08 (50%)	0.019	5.53	0.427	633.93	

MAOC (n=279)		<i>MAOC ~ Precip + Year + Land use + (1 Shelter)</i>					0.307	611.12	Year (0.193, 1.70), Precip (0.021, 6.97), Land use (<0.001, 30.85)
Traits	Rdiam * Land Use	Current	0.032 (6 yrs), -0.25 (2 yrs)	0.024	5.13	0.317	621.32		
	log(SM) * Land Use	Current	0.045 (6 yrs), -0.24 (2 yrs)	0.012	6.42	0.327	619.65		
Functional Groups	Annual Grasses * Land Use	Current	-0.16 (6 yrs), 0.11 (2 yrs)	0.004	8.49	0.329	616.98		
Diversity	Sp. Evenness * Precip	Prior	-0.13 (125%), -0.05 (50%)	0.031	4.68	0.326	619.92		
	Sp. Evenness * Land Use	Current	-0.05 (6 yrs), -0.16 (2 yrs)	0.011	6.63	0.333	615.08		
WEOC (n=194)		<i>WEOC ~ Precip + Land use + (1 Shelter)</i>					0.006	1640.88	Precip (0.758, 0.10), Land use (0.852, 0.04)
Traits	Asat	Prior	4.79	0.031	4.75	0.027	1629.43		
Functional Groups	Annual Forbs	Current	8.33	0.046	4.05	0.022	1630.59		
Diversity	Species Richness	Prior	2.96	0.030	4.76	0.029	1629.35		

Functional group and species diversity effects of each soil C pool

In linear mixed effect models including percent cover for each plant functional group, all groups had some significant correlation with a soil C pool, but most of those correlations varied across environmental conditions (Table 3). Only two functional groups were significantly correlated with soil C pools across environmental conditions: current year annual grasses were negatively correlated with TC ($p=0.024$, $F=5.12$) and current year annual forbs were positively correlated with WEOC ($p=0.031$, $F=4.75$) (Figure 4). Effect estimates are reported in Table 3. In other cases, the correlations between functional groups and soil C pools depended on the precipitation and land use history. Current year perennial forbs had a more positive correlation with MBC in 125% precipitation conditions ($p=0.039$, $F=4.29$), while prior year annual forbs had a more positive correlation with MBC in the 50% precipitation treatment ($p=<0.001$, $F=14.41$; Figure 4). Current year perennial grasses had a more positive correlation with POC in the 50%

precipitation conditions ($p=0.030$, 4.75; Figure 4). Lastly, current year annual grasses only had a positive correlation with MAOC in the newer plots with 2 years of prior shrub history ($p=0.004$, 8.49; Figure 4).

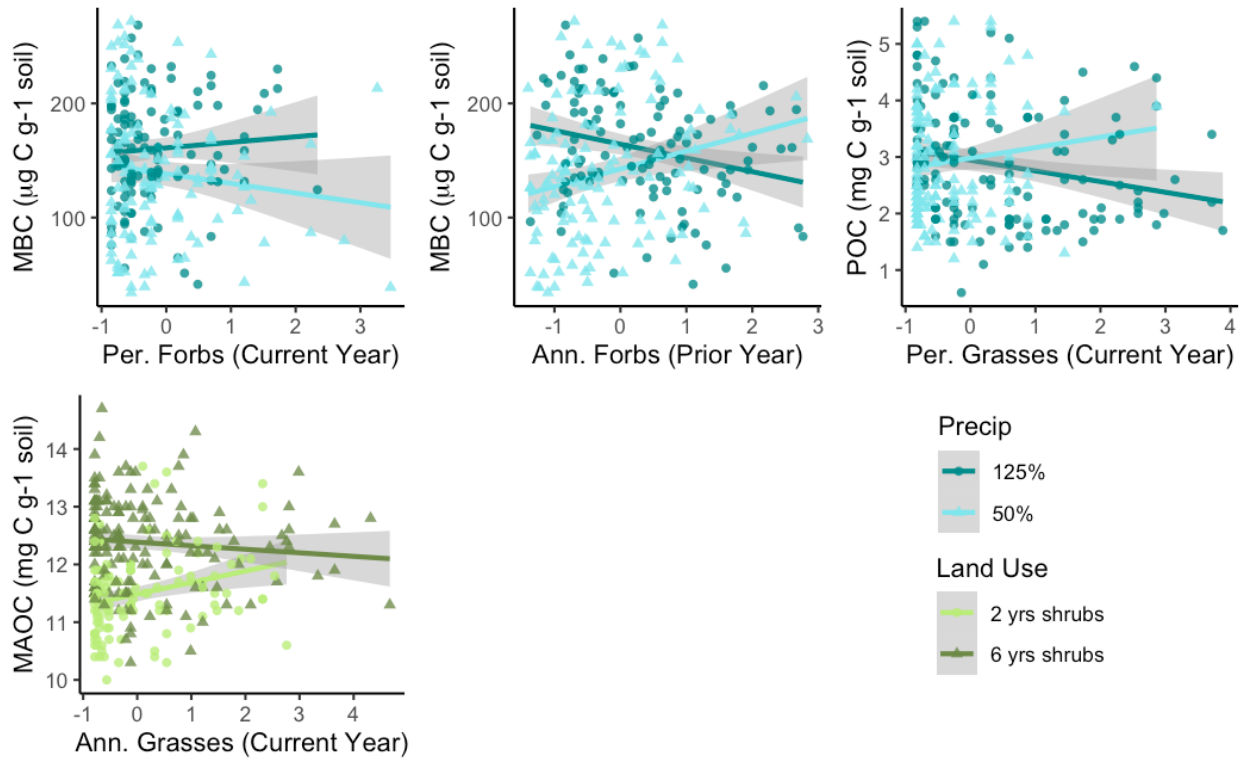


Figure 4: Species functional group correlations with microbial biomass carbon (MBC), particulate organic carbon (POC), and mineral-associated organic carbon (MAOC). Groups tested were annual (ann.) grasses and forbs, and perennial (per.) grasses and forbs.

Species diversity metrics, like functional groups, also had minor and varied effects on soil C pools. The positive correlation between WEOC and prior year species richness ($p=0.030$, $F=4.76$; Figure 5) was the only species diversity correlation found across environmental conditions. When interaction effects were tested, there were three species diversity interactions with precipitation and one with land use (Figure 5). TC was significantly affected by an interaction between precipitation and prior year species richness ($p=0.045$, $F=4.060$). In both precipitation treatments, TC had a slight negative correlation with prior year species richness. However, the magnitude of the differences between precipitation treatments was likely not

ecologically relevant (Figure 5). MBC ($p=0.028$, $F=4.89$) had a positive relationship with prior year species evenness in 125% precipitation plots and a negative relationship in the drought plots. MAOC had a similar positive correlation with prior year evenness in 125% precipitation plots, but a neutral correlation in 50% precipitation plots ($p=0.031$, $F=4.68$). Only MAOC had a significant interaction between land use and a species diversity metric. MAOC and current year species evenness had a negative correlation in plots with two years of shrub occupation and a neutral correlation in plots with six years of shrub occupation ($p=0.011$, $F=6.63$).

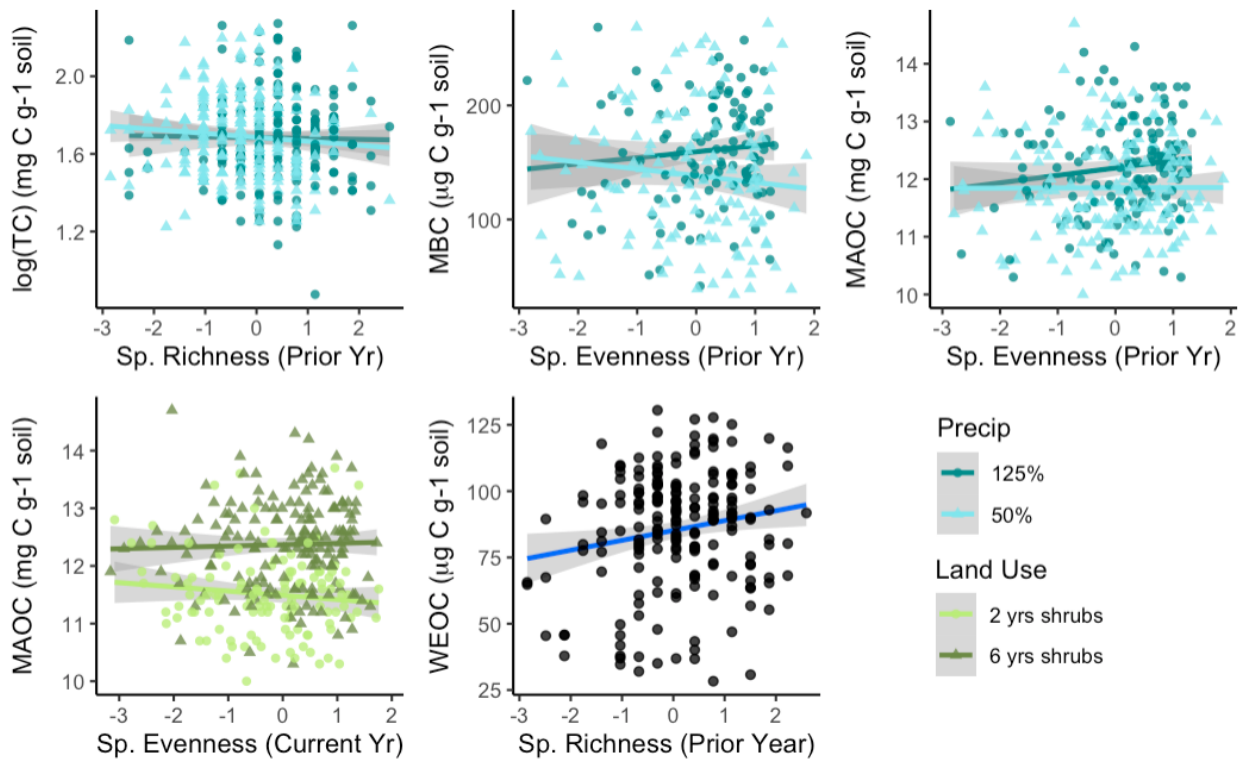


Figure 5: Significant species (sp.) diversity correlations with total carbon (TC), microbial biomass carbon (MBC), mineral-associated organic carbon (MAOC) and water extractable carbon (WEOC).

Discussion

We set out to test whether plant functional groups, functional traits or diversity metrics would have a greater effect on five different soil C pools, and whether precipitation and land use history would moderate those effects in semi-arid grasslands. We expected to see evidence of

acquisitive traits promoting WEOC, MBC and MAOC through root exudation and labile litter, and conservative traits promoting POC and TC with recalcitrant litter (De Deyn et al. 2008). Land use history was the biggest single predictor of soil C, with the duration of shrubs on the site prior to our experiment (two vs. six years) outweighing the effects of all variables calculated from our herbaceous species. Otherwise, species diversity models explained the most soil C variation, although some diversity correlations were negative. Significant individual predictors of soil C pools are explored below, but effects were often particular to each C pool and explained little of the total variation. Throughout our results, precipitation and land use history significantly influenced the effects of functional groups, functional traits, and diversity, indicating that these plant-soil relationships are highly context dependent.

Environmental treatment effects on each soil C pool

The environmental treatment variables (year, land use and precipitation) all had direct significant effects on soil carbon pools (Figure 1; Table 3). It is not surprising that year did not significantly affect TC, as this experiment was not designed to track soil C over time and we expected this pool to change gradually (Yang et al. 2019). The fact that POC showed an increase within one year shows promise for building soil C quickly via this pool, although POC is not as stable as MAOC. The accumulation of POC in non-tilled systems is one reason why the restoration of cropland to grassland is a leading strategy to increase soil C stocks (Conant et al. 2017). The increase in MBC in one year also aligns with findings that microbes respond quickly to environmental change (Gordon et al. 2008), even though the full recovery of MBC to disturbance can take years to decades (Potthoff et al. 2005, Rosenzweig et al. 2016). However, considering we detected no trait and species correlations with MBC that were consistent across

environmental conditions, our data do not directly elucidate why the significant increase in MBC occurred. It is possible the year effect on MBC is partially driven by two non-experimental factors— first, 2023 was a historically wet year (see methods), causing some additional moisture in all plots that may have stimulated microbial growth (Supplement A, Figure A4) (Liu et al. 2009). Second, the MBC samples, although frozen in both years, were processed 8 months sooner in 2023, which may have contributed to higher MBC readings despite literature showing the storage length should not matter (Rhymes et al. 2021).

Across all results, the strongest determinant of soil C was the land use history, although we cannot say exactly why carbon was higher in the plots with six years of prior shrub history. Further research would be needed to tease apart whether this effect resulted directly from the longer duration of shrubs, the break in tilling for four years while the two year shrub plots continued to get disced annually (Rosenzweig et al. 2016), or differences in the exact species combinations planted (Appendix A). Regardless, the land use history that more closely resembled croplands (four years of cover crop and two years of shrubs) had significantly lower soil C than the land use history resembling shrub-encroached grasslands (six years of shrubs and annuals). Although we expected higher soil C in the six year shrub plots based on the combination of factors above (Zavaleta and Kettleley 2006, Conant et al. 2017), it is worth noting what a strong, persistent effect this treatment had in four of the five soil C pools. The fact that we did not see an effect of land use in WEOC mirrors prior studies (Zhang et al. 2019), supporting the idea that this labile carbon pool is more connected to recent carbon inputs than older soil C (Bolan et al. 2011). We recommend researchers take extreme care in controlling land use history variation in order to elucidate the subtle changes in soil C due to traits and species diversity. However, since land use history interacted with many variables in our study, more multivariate

research is also needed to understand how the effects of certain traits, functional groups, or diversity metrics depend on the prior land use and soil C. Such research will help restoration practitioners select soil C management strategies relevant to their site context.

We expected the 125% precipitation treatment to significantly increase soil C pools through additional plant and microbial biomass growth, and root exudation. In particular, MBC and MAOC were expected to respond positively to precipitation because microbial growth is strongly dependent on soil water content, and MAOC is microbially derived (Liu et al. 2009, Lavallee et al. 2020). The effect of precipitation on MAOC supports this conclusion, but MBC results only trended in this direction ($p=0.090$, $F=3.42$, $\text{estimate}=24.64$). Since we do not have baseline soil C measures from before the experiment began, we cannot say whether MAOC increased in the 125% treatment (as in Follett et al. 2015), decreased under drought conditions or both. This detail will be important to clarify with future research since MAOC is the primary pool of stable soil C (Cotrufo and Lavallee 2022). For MBC, it is possible that microbes were bolstered in drought plots by increased root biomass allocation and exudation by plants experiencing drought (Zhang and Xi 2021), which would have weakened the effect of the precipitation treatment. The lack of precipitation effects on WEOC, despite it being a labile and sensitive pool, could be due to leaching of WEOC in wetter plots below our 0-15cm probe depth, or to the challenges of detecting changes that vary on quicker and finer scales than captured by our study (Gordon et al. 2008). Otherwise, the lack of significant differences in precipitation treatments indicate other soil C pools may be relatively stable under conditions of similar drought severity (50% average precipitation for 3 years). This result supports the continuation of grassland sequestration and storage efforts despite increasing drought with climate change.

Model comparisons within each soil C pool

Although treatment effects and AIC differences were small, models including species diversity metrics did the best at explaining TC, MBC and MAOC variation, while the environmental variables alone (year, land use and precipitation) best explained POC and no models predicted WEOC. The AIC value for the MBC prior year functional group model is also two points lower than the environmental model, but this slight improvement is usually not considered significant for AIC values (Akaike 1998). Otherwise, adding multiple traits, functional groups or functional diversity metrics to the environmental model did not predict any soil C pool better than the environmental variables alone (Table 2).

We did not find the expected positive contribution of species diversity to soil C pools (Chen et al. 2020). Rather, species diversity correlations varied with environmental conditions. The significance of TC's relationship to prior year species diversity is hard to decipher, as species diversity metrics were not individually correlated to TC (Table 3), and only showed weak interaction effects with precipitation (Figure 5). Regardless, the slight negative effect of prior year species richness in both precipitation treatments fails to support the theory of species complementarity in diverse mixes (Loreau and Hector 2001). However, we may need more time to show clearer relationships between the TC pool and the variables we tested, since TC changes slowly when plant communities are first established and the slight variations in trait and species composition are less extreme than differences in land use history (Conant et al. 2017, Yang et al. 2019).

We expected our results to align with studies showing that diverse plant communities support MBC through diverse and consistent food resources and overlapping canopies that retain more consistent soil moisture (Lange et al. 2015, Thakur et al. 2015). Rather, it seems that the

correlation between evenness and MBC depended on precipitation, being negative in dry plots and neutral in 125% precipitation plots (Figure 5). The negative effect of both leaf nitrogen content (LNC) (Figure 2) and evenness on MBC in dry plots may indicate that under water-stressed conditions, species with an acquisitive resource strategy outcompete microbes for scarce water – or at least they fail to support microbes through the water stress (Dijkstra et al. 2010). Conversely, the more positive correlations with LNC and evenness in 125% precipitation plots shows that under well-watered conditions, labile and diverse plants tissues from the prior year may benefit microbes, in line with prior research (Lange et al. 2015, Poirier et al. 2018).

Although the lowest model AIC for MAOC was found in the current year species diversity model, this is only one point lower than the environmental model, indicating that current species diversity is a weak predictor overall and may be no better than the environmental variables alone. The significance of this model seems to come from the fact that MAOC had a slight negative correlation with current year species evenness in plots with two years of prior shrubs and a neutral response in plots with a six year land use history of shrubs (Figure 5). R_{diam} and $\log(SM)$ correlate positively with current year evenness, and are also negatively correlated with MAOC (Figure 3), suggesting that these traits could be driving this pattern (effects of these are traits are discussed below). Similarly to MBC, MAOC showed a positive correlation with prior year species evenness only in 125% precipitation plots (Figure 5). This parallel relationship could be due to the fact that MAOC is primarily derived from microbial activity (Lavalley et al. 2020). In these wetter plots with higher percent plant cover (Appendix A), diverse and abundant aboveground litter might be converted more quickly by microbes from POC to MAOC (Follett et al. 2015).

Despite finding some individual correlations between plant variables, POC, and WEOC, models including multiple traits, functional groups or diversity metrics generally failed to predict these pools. Here, we should point out that WEOC had the lowest sample size and was only measured in one year, decreasing our ability to detect correlations with that pool. Otherwise, functional group models may have performed poorly since each soil C pool only correlated weakly with a subset of the four functional groups (Figure 4), and even species within the same functional group may have various effects on soil (Bezemer et al. 2006). Functional trait ecology aims to address this issue by summarizing complex plant communities into a few core mechanistic traits. However, our functional trait models failed to achieve that goal in that the functional trait models performed worse than functional group models except in one case—for MAOC in the current year. A different selection of traits could likely improve the models, but identifying that selection is the challenge. For example, a recent review suggests that chemical and symbiotic trait measures may capture plant and soil C dynamics better than morphological and architectural traits (Poirier et al. 2018). Added to the challenge of detecting trait effects in speciose systems is that traits respond to their physical and biotic environments (Zhang and Xi 2021), so there may be some error in CWM calculations based on species averages.

Functional trait effects on each soil C pool

While multi-trait models were not the best predictors of soil C pools, some individual trait and functional diversity correlations are worth mentioning. Although root traits have been theorized to contribute more to soil C (Poirier et al. 2018), we found an equal number of correlations with aboveground and belowground traits. The slight negative correlations between TC and current year root mass fraction (RMF), and the positive correlation between WEOC and

photosynthetic rate (Asat) give some support to a growing understanding that faster growing, acquisitive plants can support soil C levels (Dynarski et al. 2020). Since high RMF plants allocate more biomass underground, there could have been a priming effect at play where well-distributed exudates from more abundant roots stimulated microbial processing and decomposition of existing soil TC stocks (Chen and Chen 2018). However, others have tied low RMF to priming before (Henneron et al. 2020). While we did expect fast growing plants to contribute to WEOC through root exudates and labile tissues (Bolan et al. 2011, Wen et al. 2021), the one-year delay in this effect indicates that prior year decaying tissues likely had a greater effect on WEOC than current year exudates (but see the positive correlation with current year annual forbs).

As discussed briefly above, MAOC correlated negatively with R_{diam} and SM plots with two years of shrub land use history. For R_{diam} , this may also support the value of resource-acquisitive plant strategies for building soil C. Some recent work places R_{diam} on a separate “collaboration gradient” in which thick roots allow plants to foster mycorrhizae associations, but we do not yet understand how that impacts soil C (Wen et al. 2021). More research is also needed into the relationship between SM and soil C. SM has been related more to dispersal strategy and drought survival (Harrison and LaForgia 2019), but may be associated with soil C in our study due to its strong correlation with R_{diam} (Pearson’s correlation= 0.76, Appendix D). Since we did not detect a correlation between these traits and POC or MBC, and these correlations were based on current year plants, it is possible that much of the carbon from plants with low R_{diam} and SM ended up in the MAOC pool via direct sorption on mineral surfaces (Sanderman and Amundson 2008, Haddix et al. 2020, Cotrufo and Lavelle 2022). The lack of trait correlations in plots with six years of prior shrubs (and higher MAOC) could indicate that a MAOC saturation effect is

dampening trait effects. However, MAOC saturation is still poorly understood and a study on the saturation points across European grasslands and forests found saturation points to be higher than the MAOC values in our study (20-50 g C kg⁻¹ soil rather than 15 g C kg⁻¹ soil) (Cotrufo et al. 2019, Cotrufo and Lavelle 2022).

MBC correlated with two other traits, but only in certain environmental conditions. We already discussed the leaf nitrogen content (LNC) and precipitation interactive correlations with MBC, and the idea microbes may grow with labile, high LNC plant tissue only when sufficient water is present. Additionally, MBC was negatively correlated to root length density (RLD) in plots with six years of shrub land use history and higher relative soil C, but positively correlated in plots with only two years of prior shrubs. We expected a higher density of roots to support more microbial habitat since more microbes are found in the rhizosphere (Cotrufo and Lavelle 2022). The two year shrub plots experienced more frequent tilling in recent history and likely had lower MBC and more potential to respond positively in high RLD plots. However, the negative correlation to RLD in six year shrub plots warrants further study. Perhaps the two land use histories selected for different species (e.g. fewer perennial grasses in 6 year shrub plots (Appendix A), or other undetected differences) that had disparate root-MBC relationships (Bezemer et al. 2006).

Moving from single traits to functional diversity correlations, POC was the only pool to correlate with functional richness and evenness (FRic and FEve). The current year FRic and precipitation interactions in POC show that functional richness may be particularly important in drought conditions (Figure 2). POC also has a more positive relationship with current year FEve in plots with only two years of prior shrub land use history (Figure 3). These patterns may be because high functional richness and evenness increase the chance of resource partitioning and

of a plot containing the trait values necessary to sustain biomass growth in more stressful (drought) or degraded (tilled and low soil C) conditions (i.e. sampling effects) (Tilman et al. 1997). A recent paper in forests found a similar trend where functional diversity had a positive effect on soil C stocks in only drier forests, supporting the extension of the stress gradient hypothesis from plant productivity to soil C stocks (Chen et al. 2022). The negative correlations with functional diversity in the plots with six years of shrub and more precipitation could also occur if more evenly diverse plant communities accelerate the processing of POC into other soil C pools in these relatively C rich plots and wet plots, respectively (Chen et al. 2020, Cotrufo and Lavellee 2022). This would align with research showing more microbial activity in functionally diverse communities (Chen et al. 2020), but we did not detect any parallel significant interaction between land use and functional diversity metrics in the MBC pool. Lastly, the fact that MBC correlates negatively to species evenness in drought conditions (Figure 5) indicates that these soil C pools may have very different relationships to diversity metrics in various environmental conditions.

Functional group and species diversity effects of each soil C pool

Every soil C pool analyzed was correlated with one or more functional group, but the amount of variation explained was low and correlations were often dependent on environmental interactions. The slight negative correlation between current year annual grasses and TC was surprising since we would expect a time lag in effects on TC. Perhaps the rapid growth of acquisitive annual grasses in spring was able to stimulate TC loss via priming effects, even if that soil C was later replenished at the end of the season (Chen and Chen 2018). WEOC correlated positively with current year annual forbs, which fits with expectations since annual forbs tend to

be resource acquisitive to escape summer drought in California (Welles and Funk 2021), potentially releasing more root exudates during the growing season (Wen et al. 2021). Moreover, dominant forbs like *Amsinkia sp.* and *Phacelia sp.* were already beginning to senesce and accumulate leaf litter at the time of soil collection, which could also contribute to WEOC in the same year (Sanderman and Amundson 2008). Perennial forbs had a slight positive correlation with MBC, but only in well-watered conditions, suggesting that the benefit of perennial forbs to microbes is sensitive to environmental conditions. This aligns with a review showing that added precipitation aids MBC growth, but MBC can be resistant to loss under drought (Zhang and Xi 2021). The opposite direction of MBC trends seen with annual forbs may reflect a tradeoff between annual and perennial forb growth strategies; annual forbs generally have a drought escape strategy that includes growing quickly and supporting microbes even in dry conditions, while conservative perennial forbs could grow slowly in drought conditions, leading to a neutral MBC effect.

POC and MAOC appeared minimally associated with functional groups except for one environment-dependent relationship for each pool. POC correlated positively to current year perennial grasses in dry plots and negatively in wet plots. The positive correlation in dry plots may be because perennial grasses are better able to grow and persist through drought with their deep root structures and conservative growth strategies (Koteen et al. 2011). The slightly negative correlation with perennial grasses in 125% precipitation plots is possibly because they may persist much later in the year than other functional groups (Koteen et al. 2011), delaying the contribution of current year tissues into the POC pool. MAOC was only slightly positively correlated with current annual grasses in two year shrub plots. Again, this supports the idea that MAOC may acquire carbon quickly from acquisitive species, but it may also be more likely to

show such a response in low soil C conditions that have not approached saturation (Cotrufo and Lavellee 2022).

Species diversity, like functional diversity, also shows several interacting effects with precipitation and land use, as well as three main effects on soil C pools. TC, MBC, and MAOC relationships with species diversity were already discussed in the model comparisons section of this discussion since species diversity models were most important for those pools. The positive correlation between WEOC and prior year species richness is the only positive main effect of any diversity metric on any soil C pool. We expected to see this result in more pools, but prior studies have shown that the beneficial effects of diversity on ecosystem functions increase over time (Meyer et al. 2016, Yang et al. 2019), so it is possible that we would detect more positive diversity effects in future years. Overall, species diversity did not have consistent effects across land use histories or precipitation treatments in several cases (Figure 5), and these complex interactions will need to be tested further to tease apart their implications.

Implications for management

The fact that two significant species diversity models, two trait correlations, and about half of the significant correlations overall were from prior year species data indicates that there is often a time lag before these tissues end up in soil C pools (Fossum et al. 2022). In fact, all pools except the most labile (WEOC) were most strongly predicted by the land use history of the plots three to four years earlier. Stronger prior year effects for some pools make sense since soils were collected during the peak growing season, and most current year tissues were still actively growing rather than decomposing into soil carbon. MBC, which can respond within a year to some site changes (Gordon et al. 2008), related more to prior year measures, supporting the

importance of decaying litter inputs and older soil organic matter for microbes (Dynarski et al. 2020). However, several correlations were related to current year variables, especially for TC and MAOC. Although we explained our caution around quick changes in TC, quick changes to MAOC support the theory of a direct MAOC formation pathway separate from microbial decomposition of POC (Haddix et al. 2020). Overall, we recommend ecologists take care when correlating ecosystem effects with only current year CWM trait values, especially in annual systems with high interannual variability in species composition. The evaluation of both current and prior tissues may elucidate important mechanisms and timelines of soil C formation.

A few limitations of our study may have hindered our ability to detect the mechanisms driving change in soil C pools. First, baseline readings of the different C pools would have aided in direct measures of change rather than correlation analyses between all explanatory and dependent variables. Rather than plants affecting soil C, it is possible that the predominant direction of effect is that preexisting soil C variation in our plots altered soil properties like soil infiltration capacity and soil fertility, which in turn influenced plant community compositions (Pérès et al. 2013, England et al. 2016, Liu et al. 2019). Although these feedbacks likely exist (Liu et al. 2019), prior research in this field has found more abundant evidence of plants influencing soil carbon in alignment with our suggested conclusions (De Deyn et al. 2008, Chen et al. 2020, Hanisch et al. 2020). Another limitation is that longer term studies are preferred for slow-changing pools like TC (Yang et al. 2019). However, other studies have detected rapid changes within the other four pools due to environmental and biotic factors (Gordon et al. 2008, Keller et al. 2021, Fossum et al. 2022). Finally, since our plots all contain a mix of species, the range of CWM trait values and functional group percent covers is inherently dampened, making the detection of trends more difficult. The inclusion of monoculture plots for each species or

functional group would aid in the detection of effects (Loreau and Hector 2001), but would not mirror the species richness naturally found in similar California grasslands.

Conclusion

Our study showed that the effects of plants on soil C were often context and C pool dependent, highlighting the importance of measuring multiple soil C pools simultaneously in our quest to understand and manage soil C dynamics. The most consistent effect across soil C pools was the higher soil C levels found in plots with a land use history of six years of shrub growth. Since land use history and precipitation also moderated the effects of other variables on soil C, we recommend that more studies incorporate environmental treatments to elucidate which carbon sequestering strategies are consistent across conditions. For example, MBC seemed to respond to species diversity only in wet conditions while POC seemed to benefit from functional diversity in dry conditions. Such interactions will be important to consider as we move into a future with less stable precipitation. In functional trait models, all pools correlated with an acquisitive trait value in at least one environmental context, aligning with a growing understanding that labile tissues can feed stable as well as labile soil C pools. The fact that variables calculated from the current and prior year vegetation were both linked to soil C pools also indicates that researchers need to carefully consider the timing of species composition, functional trait, and soil C measures to better reveal these plant-soil relationships.

Overall, our results align with both selection effects and biodiversity effects, highlighting the promise of select functional groups and traits to achieve restoration targets while still maintaining high species diversity for the cases in which it does aid in ecosystem function. While this study does not outline a simple plan for how to manage soil C, our context dependent results

help reveal why we often find conflicting results in literature regarding which traits or other biological factors contribute to soil C loss and gain. To tease out such complex mechanisms of change, we must continue testing these trends in highly controlled environments alongside more complex field trials. A full understanding of the drivers of soil C change is still in progress, and we hope further research will bring us closer to implementation of restoration and management projects that effectively sequester soil C long term.

Acknowledgements

Thank you to J. L. Funk, V. T. Eviner, C. Lazcano and V. M. Wauters for advice and review of this research. We also acknowledge undergraduate research assistants Alexis Luna, Alex Cao, Bianca Pahler, Changtong Wu, Jessica Hernandez, Madeline Do, Mara Guerin, Maria Hernandez, Noah Spierings, Rachel Menge, and Yvonne Durand for their help in soil analysis. This project was funded by the Garden Club of America Fellowship in Ecological Restoration, The Nature Conservancy Oren Pollak Student Research Grant for Grassland Science, the California Native Plant Society (CNPS) Student Research Grant Award, the CNPS Orange County Chapter Charlie O'Neill Research Grant, the California Native Grassland Society Grassland Research Awards for Student Scholarship, the Jastro-Shields Graduate Research Award, the Graduate Group in Ecology Fellowship at UC Davis, and the Russell L. Rustici Rangeland Research Endowment. There are no conflicts of interest.

Appendix A

Table A1: Plant species included in the experiment.

Species	Code	Common Name	Invasive	Absent in '22/'23	Annual forb	Perennial forb	Annual grass	Perennial grass
<i>Achillea millefolium</i>	ACHMIL	Common yarrow				X		
<i>Acmispon americanus</i>	ACMAME	Spanish lotus			X			
<i>Acmispon strigosus</i>	ACMSTR	Strigose lotus			X			
<i>Amsinckia menziesii</i>	AMSMEN	Menzies' fiddleneck			X			
<i>Asclepias fascicularis</i>	ASCFAS	Narrow leaf milkweed				X		
<i>Calandrinia ciliata</i>	CALCIL	Red maid			X			
<i>Cirsium occidentale</i>	CIROCC	Cobweb thistle				X		
<i>Deinandra fasciculata</i>	DEIFAS	Clustered tarweed			X			
<i>Eschscholzia californica</i>	ESCCAL	CA poppy			X			
<i>Grindelia camporum</i>	GRICAM	Common gumplant				X		
<i>Layia platyglossa</i>	LAYPLA	Coastal tidytips			X			
<i>Lupinus bicolor</i>	LUPBIC	Miniature lupine			X			
<i>Lupinus microcarpus</i>	LUPMIC	Chick lupine			X			
<i>Lupinus succulentus</i>	LUPSUC	Arroyo lupine			X			
<i>Melica imperfecta</i>	MELIMP	CA melic		X		X		
<i>Phacelia cicutaria</i>	PHACIC	Caterpillar phacelia			X			
<i>Plantago erecta</i>	PLAERE	Dotseed plantain			X			
<i>Ranunculus californicus</i>	RANCAL	CA buttercup				X		
<i>Sisyrinchium bellum</i>	SISBEL	Blue eyed grass		X		X		
<i>Trifolium willdenovii</i>	TRIWIL	Tomcat clover			X			
<i>Aristida purpurea</i>	ARIPUR	Purple three awn						X
<i>Bromus carinatus</i>	BROCAR	CA brome grass						X
<i>Elymus condensatus</i>	ELYCON	Giant wild rye						X
<i>Elymus glaucus</i>	ELYGLA	Blue wildrye						X
<i>Festuca microstachys</i>	FESMIC	Small fescue					X	
<i>Hordeum brachyantherum</i>	HORBRA	Meadow barley						X
<i>Hordeum intercedens</i>	HORINT	Bobtail barley					X	
<i>Koeleria macrantha</i>	KOEMAC	Prairie junegrass						X
<i>Muhlenbergia rigens</i>	MUHRIG	Deergrass		X				X
<i>Poa secunda</i>	POASEC	One sided blue grass		X				X
<i>Sporobolus airoides</i>	SPOAIR	Alkali sacaton						X
<i>Stipa pulchra</i>	STIPUL	Purple needle grass						X
<i>Bromus diandrus</i>	BROMAD	Ripgut brome	X				X	
<i>Bromus madritensis</i>	BROMAD	Foxtail brome	X				X	
<i>Festuca perennis</i>	FESPER	Italian ryegrass	X				X	

Species planted (one individual per species per plot) in prior study in 9 shelters (A-I) from 2013-2016 (Funk et al. 2021):

Invasive annuals:

Avena barbata, *Brassica nigra*, *Bromus madritensis*, *Medicago polymorpha*

Native perennial shrubs:

Artemisia californica, *Encelia californica*, *Isocoma menziesii*, *Salvia apiana*

Species planted (one individual per species per plot) in prior study in 9 shelters (A-I) from 2017-2019 (Gregory Vose, personal communication):

Malosma laurina, *Artemisia californica*, *Eriogonum fasciculatum*, *Ericameria palmeri*, and *Salvia mellifera*

Species planted (one individual per plot) in a prior study in 6 new shelters (J-O) from 2017-2019 (Atamian and Funk 2023)

Artemisia californica

Figure A1: Precipitation shelter design and treatment randomization.

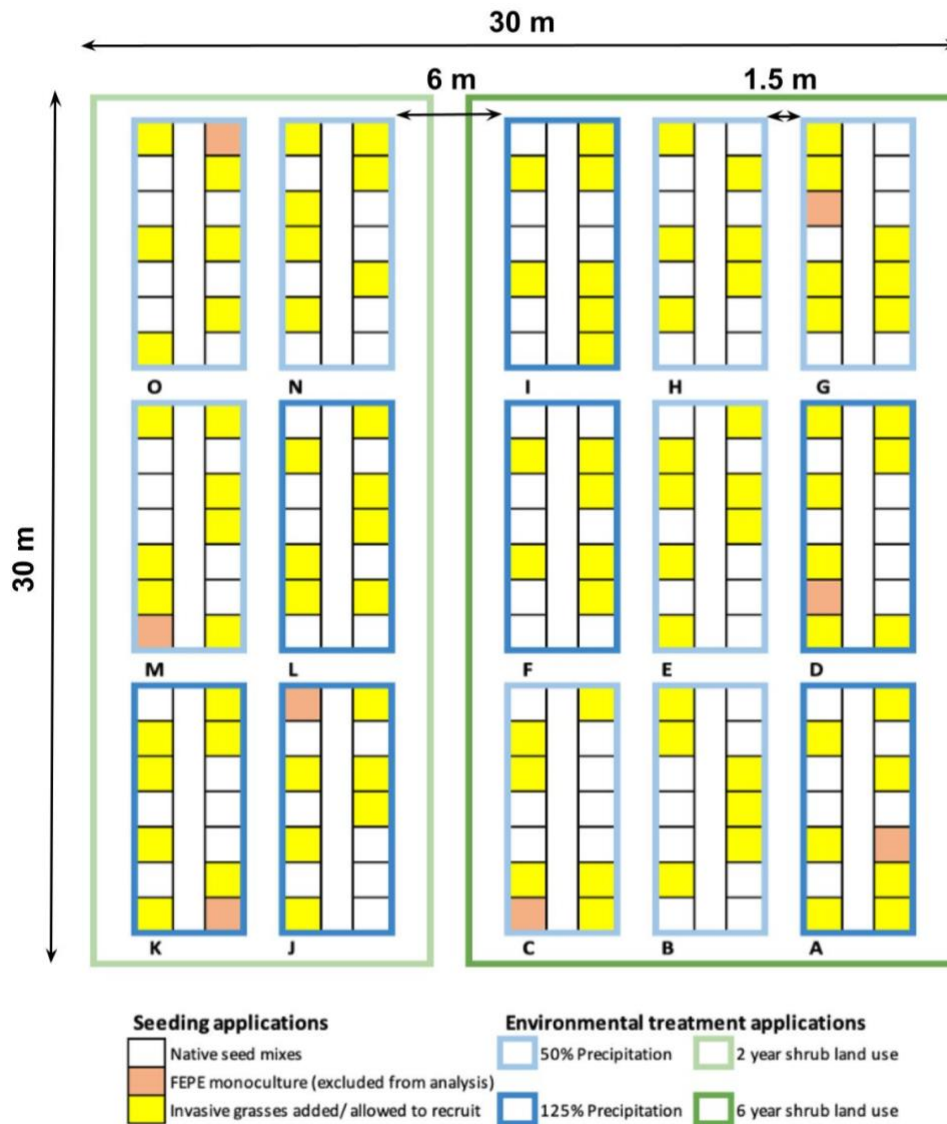


Figure A2: Plot spacing in each precipitation shelter.

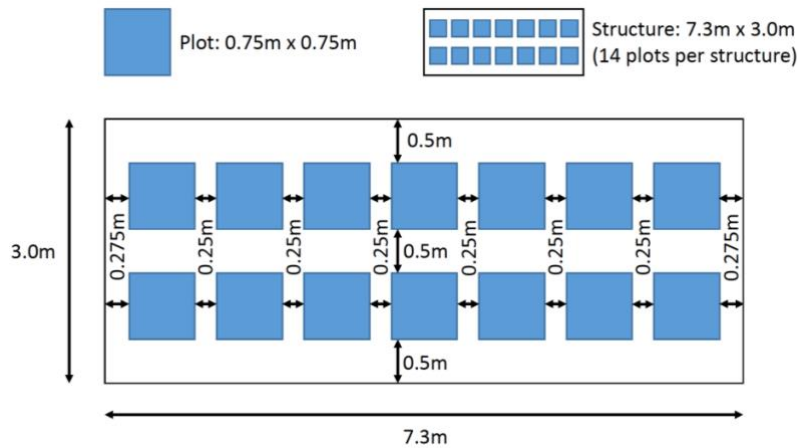


Table A2: Original sample size for each environmental treatment.

This sample size was the same for both years (2022 and 2023). There were 210 plots total but eight were planted with a monoculture (of *Festuca perennis*), which we excluded from analyses and the count below. Although soil was collected from each plot each year and analyzed for total carbon, a subset of samples was analyzed for other soil carbon pools (see methods).

Land Use	Precipitation	n (plots)	n (shelters)
6 years shrubs	50%	68	5
6 years shrubs	125%	54	4
2 years shrubs	50%	40	3
2 years shrubs	125%	40	3

Figure A3: Total percent cover and average percent cover per plot of each functional group, subdivided by the precipitation treatment (125% or 50% of average annual precipitation), land use history (two or six years of shrub occupation in the plots prior to our experiment), and year of soil sampling (2022 or 2023). NS indicates contrasts that are not significantly different. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ in post-hoc comparisons using the ggsignif package in R.

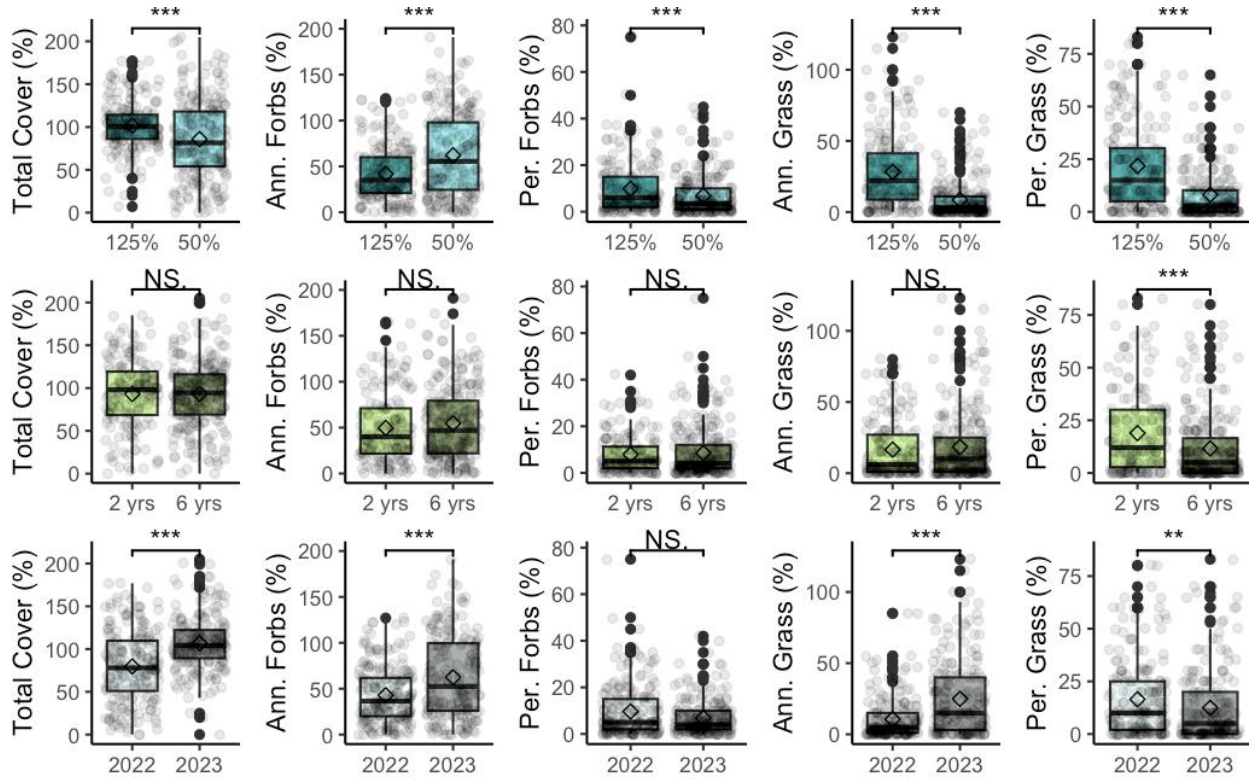


Figure A4: Average soil moisture probe readings from shelters targeted to received either 50% or 125% of annual average January-April precipitation. 2023 was a historically wet year, with heavy rains in December and early January before the shelter tarps began to be deployed. If any rain fell during days when tarps could not be deployed (around New Years in 2023), irrigation was reduced as needed to reach target January-April values.

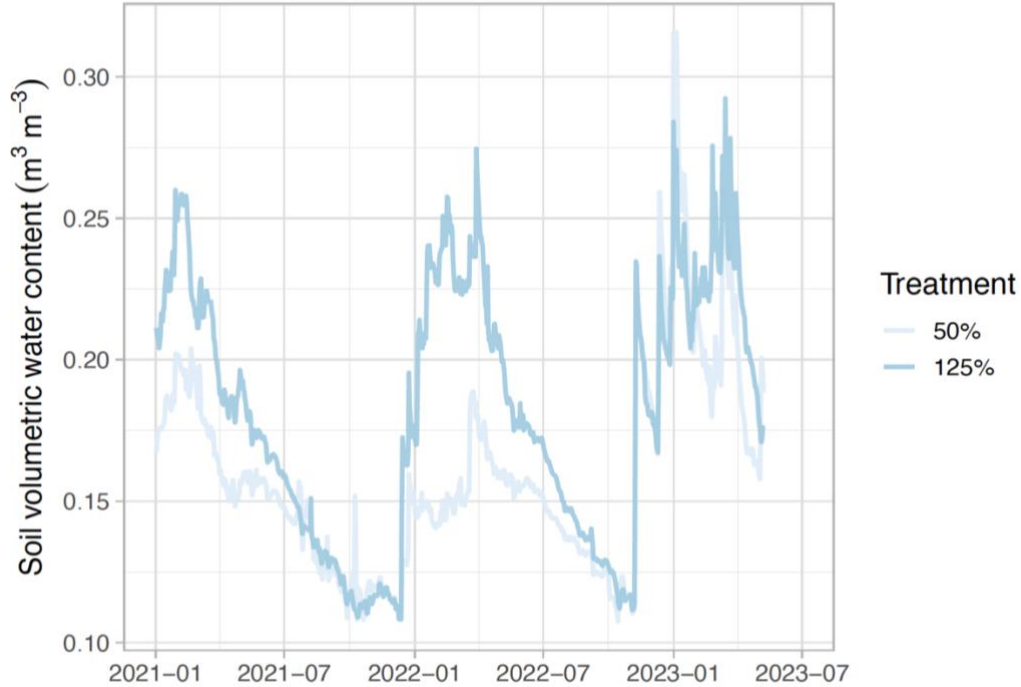
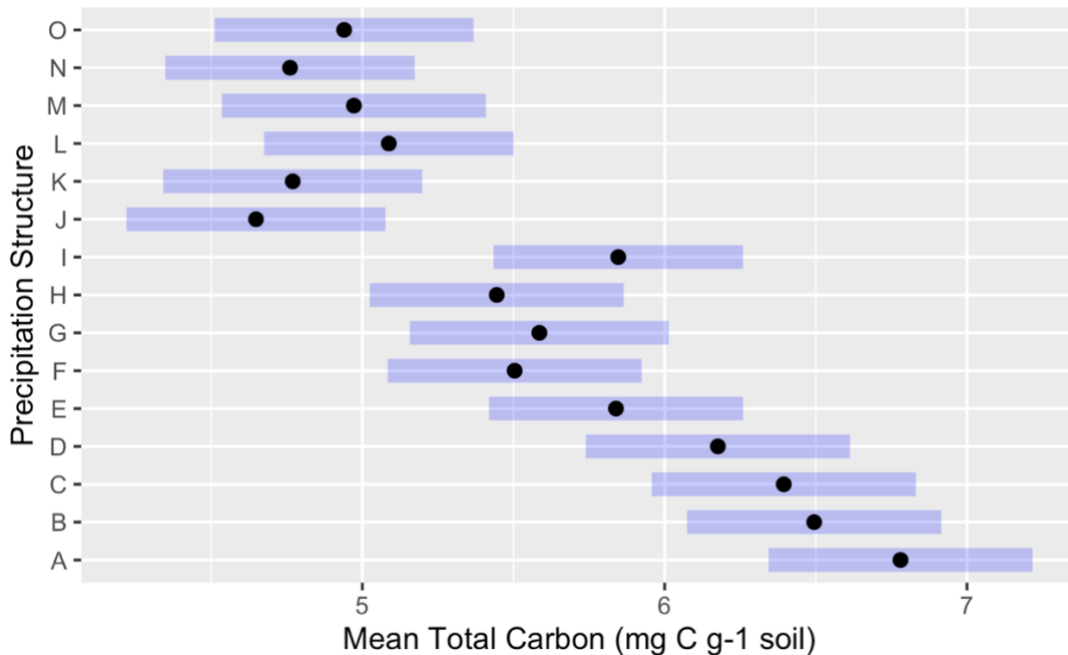


Figure A5: Mean total carbon in each precipitation shelter. Shelters A-I were included in prior experiments for six years, while shelters J-O were only included in prior experiments for two years.



Appendix B: Detailed multivariate model comparisons for Table 2.

Table B1: To make AIC values comparable, the models below are built from subsets of data that culls all NA values for that soil C measure. Therefore, some results are slightly different than they are for the full data set used in Table 3. Lowest AIC values are bolded for each soil C pool. R² values are marginal, except the second value for each null model is the conditional R².

Model Type	Model Variables	Vegetation Year	R ²	AIC (p-value)	Significant parameters (P-value, F-value)
			0.000		
TC (n=333)	Null: $\log(TC) \sim 1 + (1 / Shelter)$		0.264	-123.90	
Environmental Variables	$\log(TC) \sim Precip + Year + Land Use + (1 / Shelter)$		0.200	-139.5 (p<0.001) ***	Year (0.036, 4.40), Precip (0.947, 0.01), Land Use (<.001, 26.01)
Functional Traits (CWM)	+ (5 Traits) * (Precip + Land Use)	Current	0.230	-123.16 (p=0.682)	RMF (0.021, 5.40)
	+ (5 Traits) * (Precip + Land Use)	Prior	0.218	-118.86 (p=0.858)	-
Functional Groups (% Cover)	+ (4 Func. Groups) * (Precip + Land Use)	Current	0.222	-126.63 (p=0.469)	Annual Grass (0.006, 7.55)
	+ (4 Func. Groups) * (Precip + Land Use)	Prior	0.210	-121.87 (p=0.968)	-
Functional Diversity	+ (FRic + Feve) * (Precip + Land Use)	Current	0.214	-135.6 (p=0.231)	-
	+ (FRic + Feve) * (Precip + Land Use)	Prior	0.219	-135.15 (p=0.265)	Precip*Fric_P (0.036, 4.43)
Species Diversity	+(Evenness +Richness)* (Precip+Land Use)	Current	0.210	-133.07 (p=0.473)	Precip*Evenness (0.031, 4.70)
	+(Evenness +Richness)* (Precip+Land Use)	Prior	0.232	-141.21 (p=0.033) *	Richness (0.010, 6.66)
			0.000		
MBC (n=201)	Null: $MBC \sim 1 + (1 / Shelter)$		0.299	2141.10	
Environmental Variables	$MBC \sim Precip + Year + Land Use + (1 / Shelter)$		0.256	2104 (p<0.001) ***	Year (<0.001, 36.49), Precip (0.072, 3.91), Land Use (0.018, 7.39)
Functional Traits (CWM)	+ (5 Traits) * (Precip + Land Use)	Current	0.277	2121.2 (p=0.617)	LMA*Precip (0.039, 4.33)
	+ (5 Traits) * (Precip + Land Use)	Prior	0.337	2108.9 (p=0.049) *	RTD*Precip (0.003, 9.19)
Functional Groups (% Cover)	+ (4 Func. Groups) * (Precip + Land Use)	Current	0.287	2113.4 (p=0.265)	Perennial Forb*Precip (0.013, 6.30)
	+ (4 Func. Groups) * (Precip + Land Use)	Prior	0.317	2102.3 (p=0.012) *	Annual Forb*Precip (0.001, 11.15)
Functional Diversity	+ (FRic + Feve) * (Precip + Land Use)	Current	0.268	2110.8 (p=0.515)	-
	+ (FRic + Feve) * (Precip + Land Use)	Prior	0.257	2112 (p=0.675)	-

Species Diversity	+(Evenness +Richness)* (Precip+Land Use)	Current	0.278	2108.5 (p=0.276)	-
	+(Evenness +Richness)* (Precip+Land Use)	Prior	0.290	2102.2 (p= 0.032) *	Evenness (0.016, 5.91)
POC (n=236)	Null: $POC \sim 1 + (1 / Shelter)$		0.000 0.552	553.43	
Environmental Variables	$POC \sim Precip + Year + Land Use + (1 / Shelter)$		0.434	532.97 (p< 0.001) ***	Year (0.034, 4.57), Precip (0.888, 0.02), Land Use (<0.001, 38.16)
Functional Traits (CWM)	+ (5 Traits) * (Precip + Land Use)	Current	0.452	548.13 (p=0.462)	-
	+ (5 Traits) * (Precip + Land Use)	Prior	0.440	553.56 (p=0.854)	-
Functional Groups (% Cover)	+ (4 Func. Groups) * (Precip + Land Use)	Current	0.437	537.89 (p=0.839)	-
	+ (4 Func. Groups) * (Precip + Land Use)	Prior	0.465	549.13 (p=0.087)	Perennial Grass (0.026, 5.06), Annual Forb*Precip (0.028, 4.92)
Functional Diversity	+ (FRic + Feve) * (Precip + Land Use)	Current	0.448	535.11 (p=0.131)	-
	+ (FRic + Feve) * (Precip + Land Use)	Prior	0.433	542.46 (p=0.867)	-
Species Diversity	+(Evenness +Richness)* (Precip+Land Use)	Current	0.452	537.27 (p=0.0260)	-
	+(Evenness +Richness)* (Precip+Land Use)	Prior	0.433	542.1 (p=0.824)	-
MAOC (n=246)	Null: $MAOC \sim 1 + (1 / Shelter)$		0.000 0.393	547.61	
Environmental Variables	$MAOC \sim Precip + Year + Land Use + (1 / Shelter)$		0.324	528.71 (p<0.001) ***	Year (0.353, 0.87), Precip (0.006, 11.12), Land Use (<0.001, 39.29)
Functional Traits (CWM)	+ (5 Traits) * (Precip + Land Use)	Current	0.375	531.68 (p=0.028) *	RTD (0.037, 4.36), RMF* Precip (0.038, 4.37), Rdiam* LandUse (0.034, 4.54)
	+ (5 Traits) * (Precip + Land Use)	Prior	0.340	548.33 (p=0.795)	-
Functional Groups (% Cover)	+ (4 Func. Groups) * (Precip + Land Use)	Current	0.351	538.3 (p=0.217)	Annual Grass*LandUse (0.007, 7.334)
	+ (4 Func. Groups) * (Precip + Land Use)	Prior	0.352	537.24 (p=0.276)	-
Functional Diversity	+ (FRic + Feve) * (Precip + Land Use)	Current	0.341	531.14 (p=0.144)	-
	+ (FRic + Feve) * (Precip + Land Use)	Prior	0.323	538.78 (p=0.922)	-
Species Diversity	+(Evenness +Richness)* (Precip+Land Use)	Current	0.355	527.63 (p= 0.042) *	Evenness*LandUse (0.025, 5.094)
	+(Evenness +Richness)* (Precip+Land Use)	Prior	0.339	533.49 (p=0.301)	-

WEOC (n=171)	Null: $WEOC \sim 1 + (1 / Shelter)$		0.000 0.532	1469.7	
Environmental Variables	$WEOC \sim Precip + Land Use + (1 / Shelter)$		0.006	1473.5 (p=0.911)	Precip (0.801, 0.067), Land Use (0.787, 0.076)
Functional Traits (CWM)	+ (5 Traits) * (Precip + Land Use)	Current	0.041	1492.5 (p=0.749)	-
	+ (5 Traits) * (Precip + Land Use)	Prior	0.039	1493.9 (p=0.844)	-
Functional Groups (% Cover)	+ (4 Func. Groups) * (Precip + Land Use)	Current	0.054	1482.0 (p=0.278)	Annual Forbs*Precip (0.012, 6.459)
	+ (4 Func. Groups) * (Precip + Land Use)	Prior	0.040	1482.5 (p=0.578)	-
Functional Diversity	+ (FRic + Feve) * (Precip + Land Use)	Current	0.028	1477.5 (p=0.242)	-
	+ (FRic + Feve) * (Precip + Land Use)	Prior	0.019	1482 (p=0.748)	-
Species Diversity	+(Evenness + Richness)* (Precip+Land Use)	Current	0.015	1482.5 (p=0.807)	-
	+(Evenness + Richness)* (Precip+Land Use)	Prior	0.019	1481.9 (p=0.734)	-

Appendix C

Table C1: Trait averages used for each species to calculate community weighted mean traits and functional richness and evenness. Units and definitions are in Table 1.

Species	Code	SM	LMA	N	Asat	WUE	RMF	RLD	R _{diam}	SRL	RTD
<i>Achillea millefolium</i>	ACHMIL	0.00017	38.93	5.44	20.77	8.58	0.678	0.04	0.268	138.1	0.139
<i>Acmispon americanus</i>	ACMAME	0.00364	54.48	3.29	25.03	9.73	0.516	0.093	0.481	91.1	0.061
<i>Acmispon strigosus</i>	ACMSTR	0.00139	53.76	4.7	30.86	8.27	0.522	0.041	0.454	93.7	0.067
<i>Amsinckia menziesii</i>	AMSMEN	0.0014	61.37	2.57	21.84	3.62	0.539	0.194	0.32	214.2	0.059
<i>Asclepias fascicularis</i>	ASCFAS	0.00391	32.87	4.85	20.81	6.86	0.403	0.411	0.63	38	0.09
<i>Calandrinia menziesii (ciliata)</i>	CALCIL	0.00073	59.23	3.4	21.44	6.76	0.475	0.069	0.3	212.5	0.071
<i>Cirsium occidentale</i>	CIROCC	0.00593	71.44	2.72	25.05	8.84	0.471	0.142	0.382	122.1	0.078
<i>Deinandra fasciculata</i>	DEIFAS	0.00067	37.05	4.7	32.63	8.21	0.56	0.093	0.407	161.6	0.014
<i>Eschscholzia californica</i>	ESCCAL	0.00108	35.81	6.03	38.44	8.94	0.63	0.314	0.244	527.2	0.015
<i>Grindelia camporum</i>	GRICAM	0.00198	46.04	5.8	28.38	9.63	0.742	0.023	0.435	47.5	0.143
<i>Layia platyglossa</i>	LAYPLA	0.00058	23.87	4.2	18.97	4.84	0.116	0.819	0.337	167.3	0.068
<i>Lupinus bicolor</i>	LUPBIC	0.00391	42.63	4.89	27.95	6.61	0.592	0.069	0.82	34	0.058
<i>Lupinus microcarpus</i>	LUPMIC	0.02635	48.82	2.64	25.83	8.52	0.444	0.066	1.273	13.9	0.058
<i>Lupinus succulentus</i>	LUPSUC	0.0271	61.65	1.79	15.96	6.83	0.415	0.193	0.722	44.8	0.055
<i>Phacelia cicutaria</i>	PHACIC	0.00082	57.76	1.83	10.25	3.89	0.44	0.267	0.327	375.3	0.018
<i>Plantago erecta</i>	PLAERE	0.00154	61.93	4.05	28.68	5.93	0.547	0.084	0.328	204	0.059
<i>Ranunculus californicus</i>	RANCAL	0.00226	37.48	3.49	22.99	4.38	0.464	0.276	0.425	68.9	0.11
<i>Trifolium willdenovii</i>	TRIWIL	0.00202	28.3	4.23	22.86	5.57	0.212	1.288	0.461	92.6	0.068
<i>Aristida purpurea</i>	ARIPUR	0.00121	48.23	3.28	13.39	15.28	0.382	0.007	0.268	147.2	0.122
<i>Bromus carinatus</i>	BROCAR	0.00689	45.44	4	18.91	8.54	0.454	0.077	0.315	96.2	0.135
<i>Elymus condensatus</i>	ELYCON	0.00223	35.43	5.91	22.36	11.76	0.59	0.088	0.408	98.1	0.022
<i>Elymus glaucus</i>	ELYGLA	0.00369	43.29	2.44	16.7	5.96	0.331	3.06	0.387	75.9	0.115
<i>Festuca microstachys</i>	FESMIC	0.00151	48.49	4.28	24.69	8.13	0.594	0.394	0.21	305.6	0.096

<i>Hordeum brachyantherum</i>	HORBRA	0.00288	30.61	4.04	19.78	5.5	0.403	2.714	0.379	101.6	0.089
<i>Hordeum intercedens</i>	HORINT	0.00471	42.27	3.08	18.33	12.32	0.6	0.263	0.267	195.5	0.094
<i>Koeleria macrantha</i>	KOEMAC	0.00022	40.77	3.43	12.53	4.32	0.397	0.579	0.299	205.1	0.072
<i>Sporobolus airoides</i>	SPOAIR	0.00024	39.65	3.01	28.29	11.3	0.258	0.178	0.211	289.5	0.1
<i>Stipa pulchra</i>	STIPUL	0.00485	47.1	5.37	12.91	7.14	0.53	0.097	0.357	132.7	0.019
<i>Bromus madritensis</i>	BROMAD	0.00158	37.97	4.52	19.12	7.57	0.67	0.157	0.273	280.9	0.012
<i>Festuca perennis</i>	FESPER	0.00325	47.64	3.03	20.39	7.02	0.424	0.231	0.219	228.1	0.117

Figure C1: Trait principal component analysis (PCA) created with the dataset above, overlaid with individual species. This shows the general correlations between traits in multidimensional space and the alignment of species with high values of that trait.

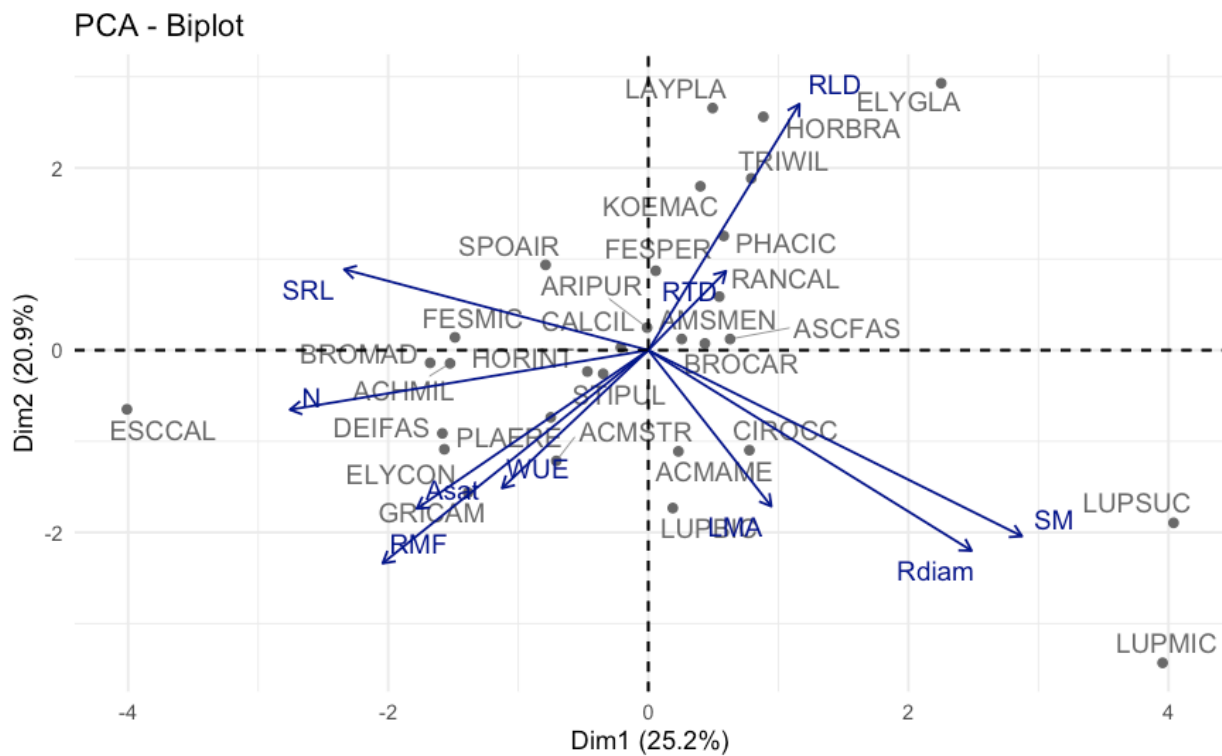


Table C2: Principal component analysis (PCA) loadings for the trait PCA analysis shown in Figure C1.

Trait	PC1	PC2	PC3	PC4
SM	0.46184893	-0.3609243	0.02134796	-0.08218373
LMA	0.15209049	-0.3030033	-0.57565467	0.17467595
N	-0.44292220	-0.1154592	0.46233984	-0.11112533
Asat	-0.28617080	-0.3074849	0.22299323	-0.19509874
WUE	-0.18077938	-0.2677840	0.11404682	0.52539929
RMF	-0.32831072	-0.4134386	-0.05113052	0.06287973
RLD	0.18668341	0.4774594	0.24178894	-0.12337547
Rdiam	0.39957360	-0.3889940	0.28896652	-0.25679403
SRL	-0.37587477	0.1568117	-0.45049352	-0.21074993
RTD	0.09607044	0.1533996	0.21012590	0.71185904

Appendix D

Figure D1: Pearson's correlation plot of soil carbon pools

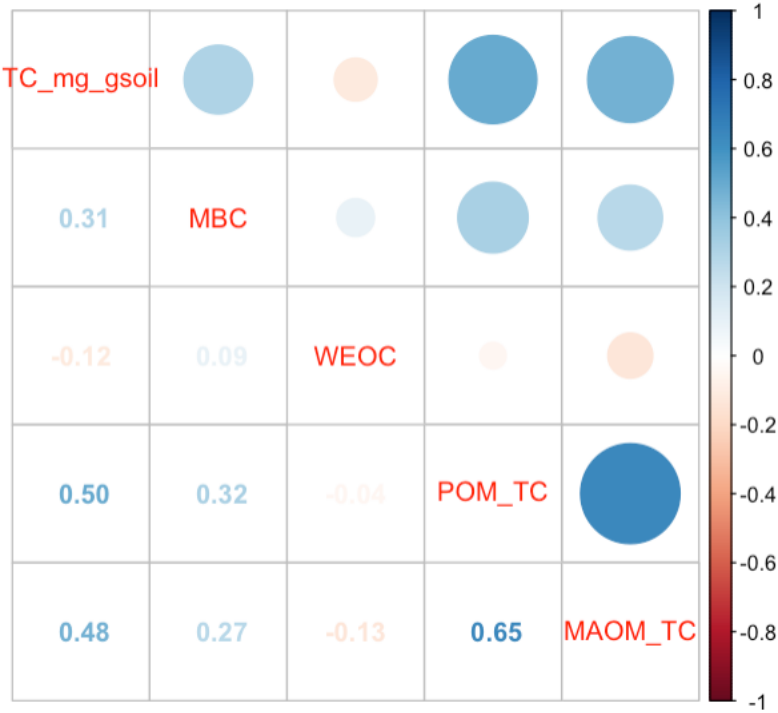
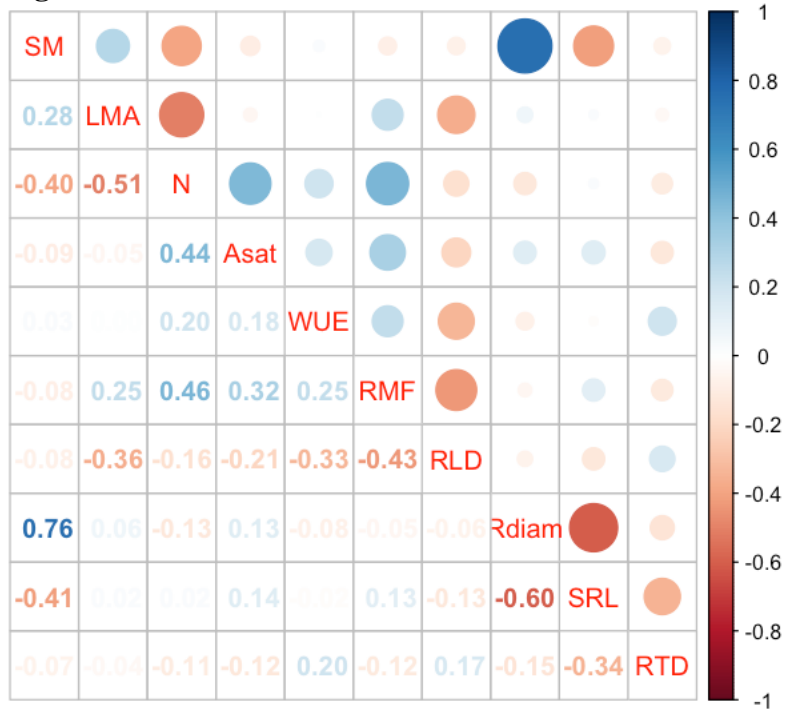


Figure D2: Pearson's correlation of functional trait measures



References

- Aerts, R., and F. S. Chapin. 2000. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Pages 1–67 *Advances in Ecological Research*. Elsevier.
- Akaike, H. 1998. Information Theory and an Extension of the Maximum Likelihood Principle. Page in E. Parzen, K. Tanabe, and G. Kitagawa, editors. *Selected Papers of Hirotugu Akaike*. Springer New York, New York, NY.
- Anacker, B. L., T. R. Seastedt, T. M. Halward, and A. L. Lezberg. 2021. Soil carbon and plant richness relationships differ among grassland types, disturbance history and plant functional groups. *Oecologia* 196:1153–1166.
- Atamian, H. S., and J. L. Funk. 2023. Physiological and transcriptomic responses of two *Artemisia californica* populations to drought: implications for restoring drought-resilient native communities. *Global Ecology and Conservation* 43:e02466.
- Bai, Z. G., D. L. Dent, L. Olsson, and M. E. Schaepman. 2008. *Global Assessment of Land Degradation and Improvement 1. Identification by remote sensing*. ISRIC, World Soil Information, Wageningen, The Netherlands.
- Berg, N., and A. Hall. 2015. Increased Interannual Precipitation Extremes over California under Climate Change. *Journal of Climate* 28:6324–6334.
- Bezemer, T. M., C. S. Lawson, K. Hedlund, A. R. Edwards, A. J. Brook, J. M. Igual, S. R. Mortimer, and W. H. Van Der Putten. 2006. Plant species and functional group effects on abiotic and microbial soil properties and plant-soil feedback responses in two grasslands. *Journal of Ecology* 94:893–904.
- Billings, S. A., K. Lajtha, A. Malhotra, A. A. Berhe, M. -A. Graaff, S. Earl, J. Fraterrigo, K. Georgiou, S. Grandy, S. E. Hobbie, J. A. M. Moore, K. Nadelhoffer, D. Pierson, C. Rasmussen, W. L. Silver, B. N. Sulman, S. Weintraub, and W. Wieder. 2021. Soil organic carbon is not just for soil scientists: measurement recommendations for diverse practitioners. *Ecological Applications* 31.
- Bolan, N. S., D. C. Adriano, A. Kunhikrishnan, T. James, R. McDowell, and N. Senesi. 2011. *Dissolved Organic Matter*. Pages 1–75 *Advances in Agronomy*. Elsevier.
- Booker, K., L. Huntsinger, J. W. Bartolome, N. F. Sayre, and W. Stewart. 2013. What can ecological science tell us about opportunities for carbon sequestration on arid rangelands in the United States? *Global Environmental Change* 23:240–251.
- Butenschoen, O., S. Scheu, and N. Eisenhauer. 2011. Interactive effects of warming, soil humidity and plant diversity on litter decomposition and microbial activity. *Soil Biology and Biochemistry* 43:1902–1907.
- Butterfield, B. J., and K. N. Suding. 2013. Single-trait functional indices outperform multi-trait indices in linking environmental gradients and ecosystem services in a complex landscape. *Journal of Ecology* 101:9–17.
- Cardinale, B. J., J. E. Duffy, A. Gonzalez, D. U. Hooper, C. Perrings, P. Venail, A. Narwani, G. M. Mace, D. Tilman, D. A. Wardle, A. P. Kinzig, G. C. Daily, M. Loreau, J. B. Grace, A. Larigauderie, D. S. Srivastava, and S. Naeem. 2012. Biodiversity loss and its impact on humanity. *Nature* 486:59–67.
- Carlucci, M. B., P. H. S. Brancalion, R. R. Rodrigues, R. Loyola, and M. V. Cianciaruso. 2020. Functional traits and ecosystem services in ecological restoration. *Restoration Ecology* 28:1372–1383.

- Chen, X., and H. Y. H. Chen. 2018. Global effects of plant litter alterations on soil CO₂ to the atmosphere. *Global Change Biology* 24:3462–3471.
- Chen, X., H. Y. H. Chen, C. Chen, Z. Ma, E. B. Searle, Z. Yu, and Z. Huang. 2020. Effects of plant diversity on soil carbon in diverse ecosystems: a global meta-analysis. *Biological Reviews* 95:167–183.
- Chen, X., M. Hisano, A. R. Taylor, and H. Y. H. Chen. 2022. The effects of functional diversity and identity (acquisitive versus conservative strategies) on soil carbon stocks are dependent on environmental contexts. *Forest Ecology and Management* 503:119820.
- Conant, R. T., C. E. P. Cerri, B. B. Osborne, and K. Paustian. 2017. Grassland management impacts on soil carbon stocks: a new synthesis. *Ecological Applications* 27:662–668.
- Conant, R. T., S. M. Ogle, E. A. Paul, and K. Paustian. 2011. Measuring and monitoring soil organic carbon stocks in agricultural lands for climate mitigation. *Frontiers in Ecology and the Environment* 9:169–173.
- Conti, G., and S. Díaz. 2013. Plant functional diversity and carbon storage - an empirical test in semi-arid forest ecosystems. *Journal of Ecology* 101:18–28.
- Corbin, J. D., C. M. D’Antonio, A. W. Dyer, and Mark. R. Stromberg. 2007. Introduction. Pages 1–4 in Mark. R. Stromberg, J. D. Corbin, and C. M. D’Antonio, editors. *California grasslands: Ecology and management*. University of California Press, Berkeley, California.
- Cotrufo, M. F., and J. M. Lavelle. 2022. Soil organic matter formation, persistence, and functioning: A synthesis of current understanding to inform its conservation and regeneration. Pages 1–66 *Advances in Agronomy*. Elsevier.
- Cotrufo, M. F., M. G. Ranalli, M. L. Haddix, J. Six, and E. Lugato. 2019. Soil carbon storage informed by particulate and mineral-associated organic matter. *Nature Geoscience* 12:989–994.
- Craine, J. M., W. G. Lee, W. J. Bond, R. J. Williams, and L. C. Johnson. 2005. Environmental constraints on a global relationship among leaf and root traits of grasses. *Ecology* 86:12–19.
- De Deyn, G. B., J. H. C. Cornelissen, and R. D. Bardgett. 2008. Plant functional traits and soil carbon sequestration in contrasting biomes. *Ecology Letters* 11:516–531.
- De Vries, F. T., C. Brown, and C. J. Stevens. 2016. Grassland species root response to drought: consequences for soil carbon and nitrogen availability. *Plant and Soil* 409:297–312.
- Díaz, S., S. Lavorel, F. De Bello, F. Quétier, K. Grigulis, and T. M. Robson. 2007. Incorporating plant functional diversity effects in ecosystem service assessments. *Proceedings of the National Academy of Sciences* 104:20684–20689.
- Dijkstra, F. A., J. A. Morgan, D. Blumenthal, and R. F. Follett. 2010. Water limitation and plant inter-specific competition reduce rhizosphere-induced C decomposition and plant N uptake. *Soil Biology and Biochemistry* 42:1073–1082.
- Dondini, M., M. Martin, C. De Camillis, A. Uwizeye, J.-F. Soussana, T. Robinson, and H. Steinfeld. 2023. Global assessment of soil carbon in grasslands. Dondini, M., Martin, M., De Camillis, C., Uwizeye, A., Soussana, J.-F., Robinson, T. & Steinfeld, H., FAO.
- Dynarski, K. A., D. A. Bossio, and K. M. Scow. 2020. Dynamic Stability of Soil Carbon: Reassessing the “Permanence” of Soil Carbon Sequestration. *Frontiers in Environmental Science* 8:514701.
- Eastburn, D. J., A. T. O’Geen, K. W. Tate, and L. M. Roche. 2017. Multiple ecosystem services in a working landscape. *PLOS ONE* 12:e0166595.

- England, J. R., K. I. Paul, S. C. Cunningham, D. B. Madhavan, T. G. Baker, Z. Read, B. R. Wilson, T. R. Cavagnaro, T. Lewis, M. P. Perring, T. Herrmann, and P. J. Polglase. 2016. Previous land use and climate influence differences in soil organic carbon following reforestation of agricultural land with mixed-species plantings. *Agriculture, Ecosystems & Environment* 227:61–72.
- Follett, R. F., and D. A. Reed. 2010. Soil Carbon Sequestration in Grazing Lands: Societal Benefits and Policy Implications. *Rangeland Ecology & Management* 63:4–15.
- Follett, R. F., C. E. Stewart, E. G. Pruessner, and J. M. Kimble Retired. 2015. Great Plains Climate and Land-Use Effects on Soil Organic Carbon. *Soil Science Society of America Journal* 79:261–271.
- Fornara, D. A., and D. Tilman. 2008. Plant functional composition influences rates of soil carbon and nitrogen accumulation. *Journal of Ecology* 96:314–322.
- Fossum, C., K. Y. Estera-Molina, M. Yuan, D. J. Herman, I. Chu-Jacoby, P. S. Nico, K. D. Morrison, J. Pett-Ridge, and M. K. Firestone. 2022. Belowground allocation and dynamics of recently fixed plant carbon in a California annual grassland. *Soil Biology and Biochemistry* 165:108519.
- Funk, J. L., E. E. Cleland, K. N. Suding, and E. S. Zavaleta. 2008. Restoration through reassembly: plant traits and invasion resistance. *Trends in Ecology & Evolution* 23:695–703.
- Funk, J. L., M. K. Hoffacker, and V. Matzek. 2015. Summer irrigation, grazing and seed addition differentially influence community composition in an invaded serpentine grassland: Community assembly in serpentine grassland. *Restoration Ecology* 23:122–130.
- Funk, J. L., J. E. Larson, G. M. Ames, B. J. Butterfield, J. Cavender-Bares, J. Firn, D. C. Laughlin, A. E. Sutton-Grier, L. Williams, and J. Wright. 2017. Revisiting the Holy Grail: using plant functional traits to understand ecological processes. *Biological Reviews* 92:1156–1173.
- Funk, J. L., J. E. Larson, M. D. Blair, M. A. Nguyen, and B. J. Rivera. in review. Drought response in herbaceous plants: a test of the integrated framework of plant form and function. *Functional Ecology*.
- Funk, J. L., J. E. Larson, and J. Ricks-Oddie. 2021. Plant traits are differentially linked to performance in a semiarid ecosystem. *Ecology* 102.
- Ge, X. M., J. P. Scholl, U. Basinger, T. E. Huxman, and D. L. Venable. 2019. Functional trait trade-off and species abundance: insights from a multi-decadal study. *Ecology Letters* 22:583–592.
- Gordon, H., P. M. Haygarth, and R. D. Bardgett. 2008. Drying and rewetting effects on soil microbial community composition and nutrient leaching. *Soil Biology and Biochemistry* 40:302–311.
- Gould, I. J., J. N. Quinton, A. Weigelt, G. B. De Deyn, and R. D. Bardgett. 2016. Plant diversity and root traits benefit physical properties key to soil function in grasslands. *Ecology Letters* 19:1140–1149.
- Grime, J. P. 1998. Benefits of plant diversity to ecosystems: immediate, filter and founder effects. *Journal of Ecology* 86:902–910.
- Haddix, M. L., E. G. Gregorich, B. L. Helgason, H. Janzen, B. H. Ellert, and M. Francesca Cotrufo. 2020. Climate, carbon content, and soil texture control the independent formation and persistence of particulate and mineral-associated organic matter in soil. *Geoderma* 363:114160.

- Haney, R. L., Alan. J. Franzluebbers, Virginia. L. Jin, M.-Vaughn. Johnson, Elizabeth. B. Haney, Mike. J. White, and Robert. D. Harmel. 2012. Soil Organic C:N vs. Water-Extractable Organic C:N. *Open Journal of Soil Science* 02:269–274.
- Haney, R. L., E. B. Haney, D. R. Smith, R. D. Harmel, and M. J. White. 2018. The soil health tool—Theory and initial broad-scale application. *Applied Soil Ecology* 125:162–168.
- Hanisch, M., O. Schweiger, A. F. Cord, M. Volk, and S. Knapp. 2020. Plant functional traits shape multiple ecosystem services, their trade-offs and synergies in grasslands. *Journal of Applied Ecology* 57:1535–1550.
- Harris, J. A., R. J. Hobbs, E. Higgs, and J. Aronson. 2006. Ecological Restoration and Global Climate Change. *Restoration Ecology* 14:170–176.
- Harrison, S., and M. LaForgia. 2019. Seedling traits predict drought-induced mortality linked to diversity loss. *Proceedings of the National Academy of Sciences* 116:5576–5581.
- Henneron, L., C. Cros, C. Picon-Cochard, V. Rahimian, and S. Fontaine. 2020. Plant economic strategies of grassland species control soil carbon dynamics through rhizodeposition. *Journal of Ecology* 108:528–545.
- Hoover, D. L., and B. M. Rogers. 2016. Not all droughts are created equal: the impacts of interannual drought pattern and magnitude on grassland carbon cycling. *Global Change Biology* 22:1809–1820.
- Horwath, W. R., and E. A. Paul. 1994. Microbial Biomass. Pages 753–773 in R. W. Weaver, S. Angle, P. Bottomley, D. Bezdicek, S. Smith, A. Tabatabai, and A. Wollum, editors. SSSA Book Series. Soil Science Society of America, Madison, WI, USA.
- Keller, A. B., E. R. Brzostek, M. E. Craig, J. B. Fisher, and R. P. Phillips. 2021. Root-derived inputs are major contributors to soil carbon in temperate forests, but vary by mycorrhizal type. *Ecology Letters* 24:626–635.
- Koteen, L. E., D. D. Baldocchi, and J. Harte. 2011. Invasion of non-native grasses causes a drop in soil carbon storage in California grasslands. *Environmental Research Letters* 6:044001.
- Lange, M., N. Eisenhauer, C. A. Sierra, H. Bessler, C. Engels, R. I. Griffiths, P. G. Mellado-Vázquez, A. A. Malik, J. Roy, S. Scheu, S. Steinbeiss, B. C. Thomson, S. E. Trumbore, and G. Gleixner. 2015. Plant diversity increases soil microbial activity and soil carbon storage. *Nature Communications* 6:1–8.
- Laughlin, D. C. 2014. Applying trait-based models to achieve functional targets for theory-driven ecological restoration. *Ecology Letters* 17:771–784.
- Lavallee, J. M., J. L. Soong, and M. F. Cotrufo. 2020. Conceptualizing soil organic matter into particulate and mineral-associated forms to address global change in the 21st century. *Global Change Biology* 26:261–273.
- Liu, W., Z. Zhang, and S. Wan. 2009. Predominant role of water in regulating soil and microbial respiration and their responses to climate change in a semiarid grassland. *Global Change Biology* 15:184–195.
- Liu, Y., H. Miao, X. Chang, and G. Wu. 2019. Higher species diversity improves soil water infiltration capacity by increasing soil organic matter content in semiarid grasslands. *Land Degradation & Development* 30:1599–1606.
- Loreau, M., and A. Hector. 2001. Partitioning selection and complementarity in biodiversity experiments. *Nature* 412:72–76.
- Luong, J. C. 2022. Nonperiodic grassland restoration management can promote native woody shrub encroachment. *Restoration Ecology* 30:e13650.

- Maestre, F. T., R. M. Callaway, F. Valladares, and C. J. Lortie. 2009. Refining the stress-gradient hypothesis for competition and facilitation in plant communities. *Journal of Ecology* 97:199–205.
- Meyer, S. T., A. Ebeling, N. Eisenhauer, L. Hertzog, H. Hillebrand, A. Milcu, S. Pompe, M. Abbas, H. Bessler, N. Buchmann, E. De Luca, C. Engels, M. Fischer, G. Gleixner, A. Hudewenz, A. Klein, H. Kroon, S. Leimer, H. Loranger, L. Mommer, Y. Oelmann, J. M. Ravenek, C. Roscher, T. Rottstock, C. Scherber, M. Scherer-Lorenzen, S. Scheu, B. Schmid, E. Schulze, A. Staudler, T. Strecker, V. Temperton, T. Tschardtke, A. Vogel, W. Voigt, A. Weigelt, W. Wilcke, and W. W. Weisser. 2016. Effects of biodiversity strengthen over time as ecosystem functioning declines at low and increases at high biodiversity. *Ecosphere* 7:1–12.
- Nguyen, M. A., A. E. Ortega, K. Q. Nguyen, S. Kimball, M. L. Goulden, and J. L. Funk. 2016. Evolutionary responses of invasive grass species to variation in precipitation and soil nitrogen. *Journal of Ecology* 104:979–986.
- Palmer, M. A., R. F. Ambrose, and N. L. Poff. 1997. Ecological Theory and Community Restoration Ecology. *Restoration Ecology* 5:291–300.
- Paruelo, J. M., G. Piñeiro, G. Baldi, S. Baeza, F. Lezama, A. Altesor, and M. Oesterheld. 2010. Carbon Stocks and Fluxes in Rangelands of the Río de la Plata Basin. *Rangeland Ecology & Management* 63:94–108.
- Pérès, G., D. Cluzeau, S. Menasseri, J. F. Soussana, H. Bessler, C. Engels, M. Habekost, G. Gleixner, A. Weigelt, W. W. Weisser, S. Scheu, and N. Eisenhauer. 2013. Mechanisms linking plant community properties to soil aggregate stability in an experimental grassland plant diversity gradient. *Plant and Soil* 373:285–299.
- Poirier, V., C. Roumet, and A. D. Munson. 2018. The root of the matter: Linking root traits and soil organic matter stabilization processes. *Soil Biology and Biochemistry* 120:246–259.
- Potthoff, M., L. E. Jackson, K. L. Steenwerth, I. Ramirez, M. R. Stromberg, and D. E. Rolston. 2005. Soil Biological and Chemical Properties in Restored Perennial Grassland in California. *Restoration Ecology* 13:61–73.
- Rees, R., and J. Parker. 2005. Filtration increases the correlation between water extractable organic carbon and soil microbial activity. *Soil Biology and Biochemistry* 37:2240–2248.
- Reich, P. B., D. Tilman, S. Naeem, D. S. Ellsworth, J. Knops, J. Craine, D. Wedin, and J. Trost. 2004. Species and functional group diversity independently influence biomass accumulation and its response to CO₂ and N. *Proceedings of the National Academy of Sciences* 101:10101–10106.
- Rhymes, J. M., I. Cordero, M. Chomel, J. M. Lavalley, A. L. Straathof, D. Ashworth, H. Langridge, M. Semchenko, F. T. de Vries, D. Johnson, and R. D. Bardgett. 2021. Are researchers following best storage practices for measuring soil biochemical properties? *SOIL* 7:95–106.
- Roscher, C., J. Schumacher, A. Lipowsky, M. Gubsch, A. Weigelt, S. Pompe, O. Kolle, N. Buchmann, B. Schmid, and E.-D. Schulze. 2013. A functional trait-based approach to understand community assembly and diversity–productivity relationships over 7 years in experimental grasslands. *Perspectives in Plant Ecology, Evolution and Systematics* 15:139–149.
- Rosenzweig, S. T., M. A. Carson, S. G. Baer, and J. M. Blair. 2016. Changes in soil properties, microbial biomass, and fluxes of C and N in soil following post-agricultural grassland restoration. *Applied Soil Ecology* 100:186–194.

- Sanderman, J., and R. Amundson. 2008. A comparative study of dissolved organic carbon transport and stabilization in California forest and grassland soils. *Biogeochemistry* 89:309–327.
- Thakur, M. P., A. Milcu, P. Manning, P. A. Niklaus, C. Roscher, S. Power, P. B. Reich, S. Scheu, D. Tilman, F. Ai, H. Guo, R. Ji, S. Pierce, N. G. Ramirez, A. N. Richter, K. Steinauer, T. Strecker, A. Vogel, and N. Eisenhauer. 2015. Plant diversity drives soil microbial biomass carbon in grasslands irrespective of global environmental change factors. *Global Change Biology* 21:4076–4085.
- Tilman, D., C. L. Lehman, and K. T. Thomson. 1997. Plant diversity and ecosystem productivity: Theoretical considerations. *Proceedings of the National Academy of Sciences* 94:1857–1861.
- USDA. (n.d.). Web Soil Survey. <https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm>.
- Voroney, R. P., P. C. Brookes, and R. P. Beyaert. 2007. Soil Microbial Biomass C, N, P, and S. Pages 637–651 in E. G. Gregorich and M. R. Carter, editors. *Soil Sampling and Methods of Analysis*. Second edition. Taylor & Francis Group, LLC.
- Wang, M., N. Lu, N. An, and B. Fu. 2021. A trait-based approach for understanding changes in carbon sequestration in semi-arid grassland during succession. *Ecosystems*.
- Weigelt, A., L. Mommer, K. Andraczek, C. M. Iversen, J. Bergmann, H. Bruelheide, Y. Fan, G. T. Freschet, N. R. Guerrero-Ramírez, J. Kattge, T. W. Kuyper, D. C. Laughlin, I. C. Meier, F. Plas, H. Poorter, C. Roumet, J. Ruijven, F. M. Sabatini, M. Semchenko, C. J. Sweeney, O. J. Valverde-Barrantes, L. M. York, and M. L. McCormack. 2021. An integrated framework of plant form and function: the belowground perspective. *New Phytologist* 232:42–59.
- Welles, S. R., and J. L. Funk. 2021. Patterns of intraspecific trait variation along an aridity gradient suggest both drought escape and drought tolerance strategies in an invasive herb. *Annals of Botany* 127:461–471.
- Wen, Z., P. J. White, J. Shen, and H. Lambers. 2021. Linking root exudation to belowground economic traits for resource acquisition. *New Phytologist* 233:1620–1635.
- Yang, Y., D. Tilman, G. Furey, and C. Lehman. 2019. Soil carbon sequestration accelerated by restoration of grassland biodiversity. *Nature Communications* 10:1–7.
- Young, T. P., D. A. Petersen, and J. J. Clary. 2005. The ecology of restoration: historical links, emerging issues and unexplored realms: *Ecology of restoration*. *Ecology Letters* 8:662–673.
- Zavaleta, E. S., and L. S. Kettlely. 2006. Ecosystem change along a woody invasion chronosequence in a California grassland. *Journal of Arid Environments* 66:290–306.
- Zhang, C., and N. Xi. 2021. Precipitation Changes Regulate Plant and Soil Microbial Biomass Via Plasticity in Plant Biomass Allocation in Grasslands: A Meta-Analysis. *Frontiers in Plant Science* 12:614968.
- Zhang, Y., X. Wang, X. Wang, and M. Li. 2019. Effects of land use on characteristics of water-extracted organic matter in soils of arid and semi-arid regions. *Environmental Science and Pollution Research* 26:26052–26059.
- Zuo, X., X. Zhou, P. Lv, X. Zhao, J. Zhang, S. Wang, and X. Yue. 2016. Testing Associations of Plant Functional Diversity with Carbon and Nitrogen Storage along a Restoration Gradient of Sandy Grassland. *Frontiers in Plant Science* 7:1–11.