Carbon and Nitrogen Dynamics in Subsoils After 20 years of Added Precipitation in a Mediterranean Grassland

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Abstract. Precipitation is a major driver of ecosystem change and physiochemical characteristics of soil. Under different climate change scenarios, increased drought frequency and changing precipitation are predicted to impact Mediterranean ecosystems, including in Northern California. Subsoils are large Carbon (C) reservoirs, however, most studies investigating precipitation effects on soil organic matter (SOM) primarily focus on near-surface soils. Recent studies indicated different responses to environmental perturbation in surface (<30cm) versus deep soils (>30cm) due to important differences in physiochemical characteristics. Here, we present soil data at depth (~300cm) from a 20-year precipitation manipulation experiment. We determined changes in total elemental concentration and stable isotope composition of soil C, N, δ^{13}C, and δ^{15}N for ambient control vs. additional precipitation in the winter and spring months. The addition of winter precipitation resulted in the largest cumulative C stock (0-300cm), however there were no statistically significant changes in C stock throughout the depth profile. However, there was evidence for vertical translocation of C to deep soil layers, specifically of plant-derived C, with both winter and spring precipitation additions. The precipitation addition in winter also resulted in the highest subsoil C stock compared to the control (ambient) and spring treatments. Overall, added winter precipitation led to the best conditions for C accumulation since the added precipitation coincides with lower temperatures and improved growing conditions at our field site. This study highlights the importance of timing of precipitation events, especially with regard to deep C stocks (>1m).

Key words: Precipitation Experiment, soil carbon, soil nitrogen, precipitation seasonality

1 Introduction

Deep soil ranging from 1-3m can account for 30 to 50% of the total soil profile Carbon (C) stock (Jobbágy and Jackson, 2000), but most published studies only sample to 50cm or shallower (Yost and Hartemink, 2020). As a result, considerable uncertainty is associated with estimations of deep global soil organic carbon (SOC) stocks. Current estimates of global SOC stocks to 1m have converged to 1100-1500 Pg, but estimates to 3m depths are 2800 Pg±700 Pg (Jackson et al., 2017). There are many reasons for the lack of data in deep soils, including logistical and cost issues associated with sampling to such great depth. However, deep soils are an active C pool and not fully accounted as a potential sink or source under current and future climate change. The limited number of deep soil studies show that subsoil C storage is affected by climate change, land use, and management change. For example, the introduction of switchgrass significantly increased subsoil C stocks (Slessarev et al., 2020) while intensive agricultural management of grasslands decreased C stocks at 100cm depth in Great Britian (Ward et al., 2016). Land use change and its effects on subsoil C are also moderated by climate (Guo and Gifford, 2002). The transition from forest to pasture increased in soil C in ecosystems with a mean annual precipitation (MAP) between 2000-3000mm, but decreased soil C in ecosystems with less than 1000mm and greater than 3000mm MAP (Guo and Gifford, 2002). However, most deep SOC responses to global change can only be hypothesized due to the few manipulative experiments on deep soils (Hicks Pries et al., 2023). Given that subsoils can be affected by climate and land use change, and act as a significant reservoir of stable C, more experimental studies are needed.

Deep soils have key physical, chemical, and biological features that make them significant reservoirs of C as well as a target for long term C sequestration. Physical characteristics of deep soils include a greater bulk density and finer texture that result in greater physical protection of C (Button et al., 2022; Plante et al., 2006) and lead to lesser accessibility of soil C compounds for microbial communities at depth (Wilpiszeski et al., 2019). In terms of biological properties, there is also lesser microbial biomass in subsoils compared to the surface, though there is evidence of microbial biomass that can be reactivated with the presence of deep roots and/or mechanical disruption (Jilling et al., 2018; Min et al., 2021). Deep soils often have a greater surface area of mineral surfaces, such as Fe/Al oxyhydroxides (Mikutta et al., 2006; Rumpel and Kögel-Knabner, 2011). These mineral surfaces are important for C stabilization, and allow for protective and stabilizing associations for C compounds that would otherwise be quickly decomposed (Kleber et al., 2005; Porras et al., 2017; Schmidt et al., 2011). When this deep C stabilization takes place, older radiocarbon ages of subsoil C are often observed which is interpreted as greater residence time (McFarlane et al., 2013; Rumpel, 2004; Rumpel & Kögel-Knabner, 2011; Sollins et al., 2009). This combination of physical, chemical, and biological features demonstrates the potential of deep soils to hold significant amounts of stabilized C.

Subsoils in grassland ecosystems are particularly important to consider due to their large global land area and their ability to store large amounts of C belowground (Bai & Cotrufo, 2022; Berhe et al., 2012; Chou et al., 2008). This large source of belowground biomass is due to deeply rooting grasses, and it is estimated that sixty percent of grassland net primary productivity (NPP) is stored belowground and is more likely to be incorporated in soil organic matter (Jackson et al., 2017). This belowground NPP results in a large soil C stock, and a meta-analysis found that grasslands have approximately 43% of grassland C stock is stored from 1-3m (Jobbágy and Jackson, 2000). It has been estimated that 44% of the variability in SOC stock uncertainty is associated with spatial scale and soil profile depth, especially due to the lack of data at greater depths and this results in significant uncertainty when estimating grassland C stocks globally (Maillard et al., 2017). Sampling deeper soils to understand stabilization mechanisms and destabilization processes of C at depths greater than 1m will be key for deep grassland soils to serve as a C source or sink under climate change conditions with changing moisture and temperature regimes.

We lack measurements of physiochemical characteristics not just deep soils, but also the effects of environmental perturbation on deep soils. However, several recent studies indicated different responses to environmental perturbation in surface (<30cm) versus deep soils (>30cm) (Berhe et al., 2008; Hicks Pries et al., 2017; Min et al., 2020, 2021). Key questions remain about whether deep soil C is vulnerable to climate change and dynamics of other key nutrients at depth, like nitrogen and phosphorus. These dynamics are important because decoupling of these nutrients from each other could impact and alter C cycling. For example, increasing aridity was found to decouple key nutrients like phosphorus (Delgado-Baquerizo et al., 2013). Long term field experiments are needed to test hypotheses at the field scale about the impact of environmental perturbation on deep soils. There is still limited data on SOC concentrations with increased precipitation in grasslands (Bai and Cotrufo, 2022). A study that looked at 30 years of precipitation augmentation in a grassland ecosystem found minimal changes in bulk C or N, but did observe greater mineral associated organic matter (MAOM) in the top 30cm (Rocci et al., 2023). Overall, there are few long-term environmental manipulations and even fewer have specifically examined the impacts of long-term manipulation on deep soils. This makes the Angelo manipulation experiment, which has been ongoing for 20 years, a particularly good site to ask questions regarding changes in soil biogeochemistry with decadal scale precipitation shifts.

Previous work at the Angelo experiment has suggested important biotic feedbacks, both plant and microbial, with changing precipitation amount and seasonality. The Angelo experiment has been ongoing for 20 years, and is testing the impacts of increased precipitation combined with changing seasonality. More specifically, it is testing the impact of shifting seasonality of precipitation to the spring months (Mar-June) in a Mediterranean climate where most of the precipitation for the water year typically takes place in the winter months (Nov-Feb). This site had multiple studies occur at the 6 and 10 year mark of the experiment (Berhe et al., 2012; Cruz-Martínez et al., 2012; Hawkes et al., 2011; Suttle et al., 2007), but this study represents one of the first long term follow ups on that experiment to great depth. Previous work at the Angelo experiment has suggested that plant and fungal communities are especially sensitive to changing amount and seasonality of precipitation. More specifically, it was found that added spring precipitation caused reductions in plant diversity (Suttle et al., 2007), while fungal communities were less diverse under both winter and spring additions (Hawkes et al., 2011). Microbial communities, on the other hand, were relatively more robust and resilient to changing water conditions (Cruz-Martínez et al., 2009), however, a later study suggested that longer term studies might be missing important short term variation driven in part by rainfall fluctuation (Cruz-Martínez et al., 2012). More recent work on microbial communities at the Angelo experiment suggested that extended rainfall decreased depth based differentiation in microbial community composition (Diamond et al., 2018). Seven years of changing seasonality and amount of precipitation also has been found to affect soil biogeochemical processes, with increased winter precipitation diminishing the role of Fe/Al oxyhydroxides in C stabilization (Berhe et al., 2012). While significant work has been done at Angelo on biotic responses to changing precipitation regimes, less work has been done on soil biogeochemistry, especially at depth (>50cm).

We wanted to examine the intersection of a long-term precipitation manipulation experiment and its potential impacts on deep soils in a northern California grassland to understand whether subsoil C stocks might be affected by climate change. The objectives of this study were to determine how changes in the amount and timing of rainfall in a California grassland ecosystem affect: (a) the distribution of C stocks to 3m, (b) the chemical composition of organic matter entering soil and its distribution throughout the soil profile, and (c) the associations between inputs and C stocks. Our hypotheses were largely based on a previous study at the precipitation experiment which focused on belowground processes (Berhe et al., 2012). This study found lower C concentration with added winter precipitation, and higher C concentration with added spring precipitation. Authors also found an accumulation of easily assimilated and less decomposed SOM and reduced rates of decomposition with spring addition. Based on this, we hypothesized that (a) there would be a reduction in C stocks with winter addition. We also hypothesized that there would be an (b) accumulation of aliphatic functional groups with spring addition and (c) a stronger association between inputs and C stocks with spring addition.

2 Methods

2.1 Site Description

The Angelo Precipitation Experiment was established in a meadow at Angelo Coast Range Reserve in Mendocino Country, California (39° 44’ 21.9762” N, -123° 37’ 50.8722” W). The dominant vegetation at Angelo is a mix of *Aira* spp., *Bromus* spp, and *Briza* spp. (Foley et al., 2023). The site is at an elevation of 1,350 m.a.s.l and experiences a Mediterranean climate with wet, cool winters and warm, dry summers. At Angelo, soils are part of the Holohan-Hollowtree-Casabonne Complex, are classified as Ultic Haploxeralfs. The parent material is largely graywacke and mudstone, and derived from Cretaceous marine grey-wacke sandstones and mudstones of the Franciscan complex, and the site overlays a bedrock terrace of the South Fork Eel River (Berhe et al., 2012).

2.2 The Rainfall Addition

The Angelo Reserve rainfall manipulation experiment was established in 2000 and set up to reflect changes in rainfall patterns predicted for Northern California over the next 50-100 years by the Hadley Centre for Climate Prediction and Research (HadCM2) and the Canadian Centre for Climate Modeling and Analysis (CCM1). For this experiment, thirty-six large circular plots (70-m^2) were regularly spaced across 2.7 ha meadows. Plots were set up in random block design for three separate treatments: (1) an ambient rainfall control; (2) a winter-addition of precipitation; (3) a spring addition of precipitation. Water addition treatments were administered by adding 14-16 mm of water every third day over three months (Fig.1). For the winter treatment, this water addition was administered from January to March, and for the spring treatment, it was administered from April to June (Fig.1). This water addition results in a 20% increase over the mean annual precipitation (Suttle et al., 2007). The supplemental water for the water addition experiment was collected from a spring above the meadow and distributed evenly over the surface of each plot through a sprinkler system (Rainbird © Raincurtain^{TM}).

Plots were assigned treatments in a randomized complete block design to take spatial biases into account (Suttle et al., 2007). Within each experimental block, treatment assignment is randomized among the plots, and then re-randomized for the next block and so on. This results in each block containing a single replicate of each experimental treatment. This design maximized the likelihood of any pre-existing differences that might exist in terms of physical or biological conditions across the grassland.

2.3 Soil Sampling

In October 2020, samples were collected by Geoprobe to depth of resistance (approximately 3m) with 4 replicates per treatment (Ambient, Winter, and Spring). For all cores, samples were collected at consistent 10cm intervals (0-10, 10-20, and so on). This resulted in a total of 345 collected soil samples across all treatments and depths.

After samples were collected, they were transported in coolers with ice packs and stored in a 4℃ cold room for approximately 4 months until they could be subsampled and analyzed. Long storage times occurred due to a lack of access to laboratory facilities due to the COVID-19 pandemic and subsequent shutdown procedures. When samples could be processed, a subsample was removed from each sample, and air dried for 7 days at room temperature. Soil samples were tested for carbonates by observing the presence and degree of effervescence with a few drops of 1 M Hydrochloric acid. Following air drying, the sample was then sieved to 2mm. A further subsample from the processed air-dried sample was taken for ball milling (using a Sample Prep 8000M Ball Mill) to a homogenous particle size.

We measured a suite of physical and chemical properties of these samples, specifically bulk density, soil pH, and gravimetric water content. We collected bulk density at Angelo through Geoprobe cores and calculated C stocks with these bulk density estimates. We subsampled each depth increment to estimate water content, and then calculated the dry mass of soil in a 10cm increment. Bulk density was calculated as the mass of the dry <2mm fraction to correct for the impact of rock and root volume on soil C and N stocks (Throop et al., 2012). Soil pH was measured in a 1:1 soil:water and soil: $CaCl_2$ slurries.

2.4 Elemental and Isotopic Analyses

For elemental and isotopic analysis of C and N, soil samples were air dried, sieved to 2mm, and ground (using both a mortar and pestle and Sample Prep 8000M Ball Mill). The $\delta^{13}C$ and $\delta^{15}N$ values and elemental C and N contents of all samples were measured in the Stable Isotope Ecosystem Laboratory of (SIELO) the University of California, Merced. Briefly, samples were weighed into tin capsules and combusted in a Costech 4010 Elemental Analyzer coupled with a Delta V Plus Continuous Flow Isotope Ratio Mass Spectrometer. C and N isotope compositions were corrected for instrumental drift, mass linearity, and standardized to the international VPDB ($\delta^{13}C$) and AIR ($\delta^{15}N$) scales using the USGS 41A and USGS 40 standard reference materials. Mean $\delta^{13}C$ values for USGS 40 and 41a were (mean ± standard deviation with n indicated) -26.4±0.1‰ (n = 118) and 36.5±0.2‰ (n = 59), respectively, and mean $\delta^{15}N$ values were -4.5±0.1‰ (n = 118) and 47.5±0.1‰ (n = 59), respectively. Elemental C and N content were determined via linear regression of CO_2 and N_2 sample gas peak areas against the known C and N contents of USGS 40, USGS 41a, and Costech acetanilide. All isotope compositions are expressed in standard delta notations.

Stable isotopes are a staple tool in the realm of soil science as ecological integrators, and can be a useful tool in understanding environmental perturbations in deep soils. C3 plants in particular have a well-documented physiological response to increasing aridity that leads to high $\delta^{13}C$ values (Farquhar et al., 1989; Kohn, 2010). $\delta^{13}C$ has been shown to vary with mean annual precipitation (MAP) due to discrimination against ^{13}C in drier areas because stomata have to remain more closed to minimize water loss (Krüger et al., 2023). This interaction between precipitation and the stable isotope values of plant matter means that the inputs for formed C will be affected by climate. These altered stable isotope values could act as a potential tracer for plant inputs into soil. There is a well-documented pattern of increasing $\delta^{13}C$ values with depth; a disruption of this pattern by ^{13}C depleted plant matter inputs could be a good indicator of formed C being distributed throughout the profile. Furthermore, Diffuse Reflectance Mid-Infrared Fourier Transform spectroscopy (DRIFTS) is a complementary analysis that can characterize the chemical composition of soil C. DRIFTS can measure the vibrational frequency of ecological relevant functional groups, like aliphatic, aromatic, and amide functional groups (Mainka et al., 2021; Margenot et al., 2015; Parikh et al., 2014). Together, stable isotopes and DRIFTS can provide important information about incoming plant matter as well as microbial activity in soils.

2.5 Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS)

To measure the presence of functional groups that are important for organic matter and mineral surfaces related to soil C across our study systems, we used diffuse reflectance mid-infrared Fourier Transform spectroscopy (DRIFTS). DRIFTS measures the vibrational frequencies of functional groups in a sample, and is well suited for analyzing soils due to the minimal sample preparation needed for this technique. We performed analyses on bulk soil samples that were ball milled to a homogenous consistency to avoid interferences that could affect baselines or peak widths. We used a Bruker IFS 66v/S Spectrophotometer (Ettlingen, Germany) with a praying Mantis apparatus (Harrick Scientific, Ossining, NY) at the Nuclear Magnetic Resonance (NMR) lab at UC Merced. It is important to note that potassium bromide (KBr) was used as a background reference, but samples were not diluted with KBr. Samples were initially dried in a desiccator following homogenization to remove interference from water. Absorption was measured between 4000 and 400 cm^{-1} averaged over 300 scans with an aperture of 4mm. Functional groups for simple plant C (aliphatic C-H; λ: 2976-2898 cm^{-}), complex plant C (aromatic C=C; λ: 1550-1500 cm^{-1}), microbially derived C (amide/quinone/ketone, CO; aromatic, CC, carboxylate COO; λ: 1660-1580 cm^{-1}) were assigned following Mainka et al. (2022), also shown in Table 1 (Mainka et al., 2021; Parikh et al., 2014; Vranova et al., 2013). We excluded wavenumbers that overlap with signal from mineral compounds, specifically from 1400-400 cm^{-1}, from the analysis (Margenot et al., 2015; Parikh et al., 2014). We integrated the area under the curve in R (v4.2.1, (R Core Team, 2022) for our functional groups of interest (aliphatic, aromatic, and amide). Bounds for integration are reported in Table 1 as "range." We then normalized the area under the curve for our functional groups of interest to 100%. We also calculated ratios of simple plant C to microbial C, as well as complex plant C to microbial C. A low ratio of simple plant C to microbial C indicates microbial oxidation of plant derived C, and a high ratio of simple plant C to microbial C indicates a high supply of aliphatic plant C to soil. Additionally, a low ratio of complex plant C to microbial C indicated more microbial oxidation of plant C, while a high ratio of complex plant C to microbial C indicates a high supply of aromatic plant compounds to soil.

2.6 Statistical Methods and Model Fitting

All statistical analyses were performed using R statistical software (v4.2.1, R Core Team, 2022). Differences between treatments for C stocks were evaluated through Kruskal-Wallis test within each 10cm (Fig. 3b) or 50cm (Fig. 3a) depth interval depending on the analysis. We used a Kruskal-Wallis test combined with a Pairwise Wilcox due to it being a non-parametric statistical test. We determined that our data was non-parametric through a Shapiro Wilk test of normality. Statistical significance was evaluated using $\alpha = 0.05$.

To better understand relationships between C stock and inputs, we performed a linear regression on C stock and C:N values. While C:N values integrate many processes, there is evidence to suggest that higher C:N (>20) indicates plant inputs, while low C:N values (<8) indicate microbial inputs (Bell et al., 2014; Knicker, 2011; Nierop et al., 2001). We interpreted a high C:N as indicative of more plant inputs, while a low C:N would be indicative of more microbial inputs and decomposition.

To test the relative importance of biotic and abiotic factors on δ^{15}N and C within treatments and to account for possible nonlinear relationships, we used a hierarchical generalized additive mixed model (GAMM) (Pedersen et al., 2019). To fit the GAMM we used the "mgcv" package (Wood, 2017). GAMMs are a type of generalized linear model where the predictor is defined by a number of smooth functions of covariates. Models avoid overfitting by penalizing each smooth function, or in other words, penalizing the "wiggliness" of the fit. We also checked concurvity (the extension of collinearity to a GAMM) through the mgcv package. We fit with smooth functions based on thin plate regression splines (which are the default) and residuals approximated a "scat" or scaled t distribution, which was assessed from residuals using the package DHARMa (Hartig, 2022). In order to compare the relative importance of abiotic (depth) and biotic (DRIFTS ratios) variables, we constructed different models: one model had all terms included, one only included depth, and one only included our DRIFTS ratios of interest that indicate microbial oxidation and plant inputs. All models were compared through the Akaike Information Criterion (AIC), and the full model with all predictors and our depth model had the lowest AIC, so not all of the models run were included for parsimony.

3 Results

3.1 Variation in physical, chemical, and isotopic parameters across treatments and depths

Physical parameters such as bulk density were relatively similar across treatments (Fig. 2a-b) though there were important differences in the surface. The control treatment had the highest bulk density at 10cm (1.7±0.1 g/cm^3) while the spring and winter treatments (1.4±0.4 g/cm^3) had lower bulk density (Fig. 2a). Bulk density increased across the depth profile, and eventually converged to similar values across all treatments (~1.8-2 g/cm^3) (Fig. 2a). C (%) was greatest at the surface (0-30cm) in the control (2.91-0.71%) and winter (3.26-0.99%) treatments, but quickly dropped off to similar values at approximately 50cm (~0.3%) (Fig. 2b). N (%) was present in low concentrations (<0.2%) across the entire profile and across treatments (Fig. 2c). However, C:N varied in key ways throughout the depth profile and between treatments (Fig. 2d). While C:N was the greatest at 10cm in the control (18.7±4.09) compared to the winter (13.9 ±1.78) and spring treatments (10.9±0.96), the winter and spring treatments had elevated C:N from 30-100cm (Fig. 2d). Changes in physical and elemental parameters were largely limited to the surface, and converged past 1m in most cases.

Stable isotope values to 3m were highly variable across the depth profile and across treatments, especially around the 100 cm depth. We did not observe treatment effects from added precipitation on δ^{13}C and δ^{15}N values (Fig. 2e-f). We expected the well-documented pattern of increasing δ^{13}C values with depth in soils (Natelhoffer and Fry, 1988) to be moderated by decreased δ^{13}C of formed C due to the added precipitation from the manipulation experiment in both the winter and spring treatments. We observed slight differences in the overall distribution of δ^{13}C values in the winter and spring treatment throughout the depth profile. However, except for the low δ^{13}C values we recorded around 300 cm depth in the spring treatment plots, we did not observe any statistically significant differences in δ^{13}C values throughout the profile.

In terms of chemical parameters, differences in pH between treatments were also limited to the top 1m and converged to similar values from 1-3m (Fig. 2g-2h). However, it is important to note that the spring and winter treatments had higher pH (H_2O) compared to the control treatment to 1m. Only the spring treatment had elevated pH ($CaCl_2$), while the control and winter treatments were similar throughout the entire depth profile. The pH values in water and $CaCl_2$ indicate slightly acidic to neutral soil pH across the soil profile for all treatments.

3.2 C stocks and elemental relationships across depth increments

We found no statistically significant differences in C stock across the 3m depth profile or at the surface (top 50cm) from our Kruskal-Wallis test. All discussion of these results in this paragraph refer to trends between calculated cumulative stocks and across depth profiles due to the lack of statistical significance determined by the Kruskal-Wallis tests. We determined changes in the overall C stock across the entire depth profile of the treatment plots and observed important changes in seasonality. We found that the Winter treatment had the greatest C stock (200.5± 34.5 g/cm^2, table 2), followed by the control treatment (191.2±36.7 g/cm^2), while the spring had the smallest cumulative C stocks (171.4±13.7 g/cm^2) over the entire profile (0-300cm) (table 1). The soil C stocks sharply dropped below 50cm in all the treatment plots. Proportionally, soils from 1-3m held 35% of the C stock in the control, 33% in the winter treatment, and 37% in the spring treatment (Fig. 3). In addition, a more detailed investigation of the surface revealed greater C stocks from 0-50cm in both the Winter and control treatments (Fig. 3). There was also relatively greater C in the winter and spring treatments at 150cm. Interestingly, the winter C stock at 300cm was decreased compared to both the control and spring treatments. Overall, however, the winter treatment had the greatest overall C stock while the spring treatment had the least.

To further interrogate relationships between soil C processes and inputs at the precipitation manipulation experiment, we looked at relationships between C stock and C:N across depth increments (Fig. 4a-b). These is evidence to support that high C:N values are indicative of plant tissues (Bell et al., 2014; Nierop et al., 2001), while low C:N values are indicative of microbial byproducts such as amino acids and amino sugars (Knicker, 2011). Overall, C:N values integrate multiple processes that can be difficult to disentangle, such the balance between inputs and decomposition (Conen et al., 2007). We expected to see significant positive relationships, indicating a tight relationship between C stock and plant inputs. We did indeed see positive and significant relationships across all treatments. We found a slightly more positive relationship in the winter treatment than the control. In the Spring treatment, we saw a much narrower range in C:N values compared to the winter and control treatments. This analysis highlighted the unique distribution of both C:N and C stock in the spring treatment.

3.3 Variation in functional group chemistry across treatments

The DRIFTS spectra show differences across treatments and depths, especially areas of interest for biological OM inputs (Fig. 5). We observed shifts in functional group chemistry of SOM across treatments, both proportionally (Fig. 6) and in relationships to elemental and isotopic data (Fig 7). We calculated proportional relationships by normalizing the area under the curve for our functional groups of interest to 100%. An important observation from the DRIFTS spectra is the proportional contribution of microbially associated functional groups across all three treatments (>50%; Fig. 6a-c). Simple plant-derived organic matter (aliphatics) was approximately 30% of the total, with some slight variations while complex plant matter (aromatic) functional groups were the smallest fraction, representing about 20% of the total (Fig. 6). Furthermore, differences in simple and plant-derived OM diverged between treatments from 200-250cm, where the control treatment seemed to have the greatest aromatic or complex plant-derived OM proportionally (Fig. 6a) and also based on the complex: microbial ratio (Fig. 6e). Our DRIFTS data overall suggested that the dominant functional groups were similar across depth and treatment, and that it was largely microbially associated organic matter.

We further analyzed the relationship of the shifts in SOM functional group chemistry to elemental and isotopic data to better understand abiotic versus biotic controls on SOM storage and processing across treatments. We chose to more closely examine δ^{15}N because of it being coupled to biotic processes (Dijkstra et al., 2008; Hobbie and Ouimette, 2009), and C (%) to potentially understand relationships of biotic and abiotic factors with C storage. We fit hierarchical GAMMs to better predict both $\delta^{15}N$ (table 3a) and C concentrations (table 3b). GAMMS were fit to try and predict $\delta^{15}N$ and C(%) to better understand if $\delta^{15}N$ is actually related to our DRIFTS ratios of interest (which we interpreted as biotic factors) and C storage. Through visualizing our GAMM, we saw significant negative relationships in the winter and spring treatment for the relationship between the simple plant: microbial and $\delta^{15}N$ values (Fig. S2a). We also observed a similar pattern for relationships between complex plant: microbial and $\delta^{15}N$ values (Fig. S2b). Overall, we were able to account for greater variation for both $\delta^{15}N$ and C (%) using GAMMs. The model including all factors for C (%) performed better ($R^2_{adj} = 0.722$) than the model for $\delta^{15}N$ values ($R^2_{adj} = 0.133$). For both C concentration and $\delta^{15}N$, depth was a key factor across all treatments. Importantly, however, the simple plant: microbial DRIFTS ratio was a significant predictor of $\delta^{15}N$ for the winter treatment, and the complex plant:microbial DRIFTS ratio was a significant predictor for both the winter and spring (Table 3a).

4 Discussion

C translocation was a key mechanism in this experiment, and there was a clear impact on the distribution of C stocks and functional groups chemistry between topsoil and subsoil from adding more water to the soil profile. We saw the greatest cumulative C stocks in the winter treatment, especially compared to the spring addition. Furthermore, plant phenology in an annual grassland varies greatly from the winter months to the spring. Spring temperatures are increasing, evaporative stress is greater, and annual plants are usually reaching the end of their life span in this season. We think that greater C is accumulating in the winter treatment due to precipitation addition coinciding with lower temperatures and lesser microbial activity. In sum, what we are likely seeing is increased plant inputs in the winter treatment and greater transport to deeper layers, whereas in the spring, due to higher temperatures and greater evaporative stress, there is more gaseous loss of C, less water, and less C moved to deeper soil layers.

4.1 C translocation and accrual as a result of added precipitation

We observed clear signals of greater C translocation throughout the soil profile as a result of added precipitation, but where this C accumulated was based on seasonality. Added precipitation seemed to lead to greater transport of C throughout the profile in the winter and spring treatment especially. However, we did not see any statistically significant differences in C stocks across both the subsoil and surface C stock measurements (Figure 3a-3b). This could be due to significant variability introduced from fixed depth bulk density measurements due to changing soil volume over time (von Haden et al., 2020). We observed significant evidence for slightly higher C in the winter treatment being plant derived based on both C:N and DRIFTS data. We also saw evidence of greater biotic processing in the Spring treatment when comparing $\delta^{15}N$ to the simple plant matter: microbially associated DRIFTS ratio (Table 3) and the overall lower and more constrained C:N values (Fig. 4). We also saw positive and significant trends when we related C concentrations to the ratio of complex plant matter to microbial associated OM (Fig. S2b), highlighting a unique relationship between complex plant inputs and C especially in the winter and spring that may be related to biotic inputs. It is important to note that evidence of subsoil C accumulation in the winter treatment was only made possible by incorporating measurements from deeper than 1m, and that depth was a significant predictor in our GAMM models across all treatments (Table 3). Previous work suggests that the main sources of organic matter into subsoils are plant derived compounds (roots and root exudates), dissolved organic matter (DOM) and bioturbation (Rumpel and Kögel-Knabner, 2011). Work at the Angelo experiment has shown evidence of changing rooting patterns and greater overall biomass with increased precipitation at this site (Suttle et al., 2007). Rooting depth and root exudation rates are affected by availability of soil water, and direction of response is strongly species dependent (Li et al., 2021; Souza et al., 2023; Staszel et al., 2022). However, it has been found through $^{13}CO_2$ pulse labelling studies that drought reduces transfer of newly fixed C to microbes (Fuchslueger et al., 2014; Ruehr et al., 2009), while manipulative experiments with rainfall addition increase root C exudation rates (Li et al., 2021). A recent meta-analysis noted that decreased precipitation also slows the belowground C cycle, while precipitation increases promote nearly every aspect, such as C stock, substrate supply, microbial activity, and respiration (Abbasi et al., 2020). This is due to interactions between precipitation and biological entities, namely plants and microbes. Increased precipitation root respiration and belowground NPP are positively correlated with soil water availability, and enhance plant growth and photosynthetic rates (Heisler-White et al., 2008; Maire et al., 2015). Wetting of dry soil also has a dramatic impact on soil microbes due to increased substrate availability and reactivation of dormant microbes, yielding respiration pulses known as the Birch effect (Salazar et al., 2018; Schimel et al., 2007; Skopp et al., 1990). Overall, greater precipitation in the winter could contribute to greater root exudation in surface soils that then gets quickly fixed by soil microbes. Our results show that in the winter treatment plots, the additional precipitation in the already wet winter season likely increases root exudation, where this increased C input coincides with lower temperatures and lesser biological activity in soil. Whereas in the spring treatment, soil C is exposed to greater microbial processing. Thus, there is greater movement of this plant derived inputs moving down the profile in both the spring and winter treatments, but to greater depths in the spring treatment, likely as DOM. Recent plot-scale studies have proposed that OM formation in subsoils is linked to a complex cascade model, in which OM is sorbed, microbially processed, and remobilized in cycles as it migrates down the profile (Liebmann et al., 2020).

4.2 Biotic shifts as a result of changing precipitation amount and seasonality

Changing plant phenology throughout the growing season and changes in plant community composition could be contributing to the differences we see in C stock accrual in our treatment plots. Increased precipitation is shown to increase net primary productivity in grasslands, but alter plant community composition and reduce diversity (Song et al., 2019; Suttle et al., 2007). Furthermore, higher plant species richness is associated with increased soil organic C (Prommer et al., 2020). At the Angelo precipitation experiment, it was shown that plant community composition responses to changing precipitation were based on seasonality, with spring addition resulting in reduced plant diversity while the winter treatment maintained diversity close to the control (ambient) plots (Suttle et al., 2007). More specifically, Suttle et al. (2007) found that annual grass biomass was greater and plant richness lower with spring addition. However, the spring treatment in our experiment still accumulated C in surface soils. This could be because invasive annual plants, such as cheatgrass, have been shown to accumulate both C and N due to higher rates of root exudation according to mesocosm experiments (Morris et al., 2016). A recent meta-analysis further examined the feedback between annuals and litter and rhizosphere inputs, and found that invasive plants may support more decomposers that stimulate more nutrient release from litter (Zhang et al., 2019). The simplification of the plant community in the spring treatment plots as well as potential for greater root exudates and greater stimulation of decomposition by annual grasses at the surface could be leading to the surface C stock accumulation, we observed from 0-50cm in the winter and spring plots. Other studies have suggested that a longer and later wet season would result in significant losses of C due to increased soil respiration (Chou et al., 2008). Increases in NPP accompanied with increased gaseous C flux is consistent with what is found in larger meta-analyses, where increased precipitation stimulates plant growth and ecosystem C fluxes (Wu et al., 2011). There is still a question at the Angelo precipitation experiment of how these changes in plant community will interact with potential stimulation of gaseous C flux.

Isotope values can also be affected by interactions between plants and climatic conditions, specifically due to plant physiology responses to soil water conditions. The δ^{13}C value of C3 plants is tied to climatic regime (Krüger et al., 2023), furthermore the plants in this ecosystem are largely C3, meaning that differences in plant isotope values and formed C are likely driven by physiological responses to changing climatic regime and soil water. More specifically, there is less discrimination against the heavy isotope when plant stomata have to close more often, which is the case in arid environments (Casson and Gray, 2008; Driesen et al., 2020; Farquhar et al., 1989; Kohn, 2010; Krüger et al., 2023; Madhavan et al., 1991). Overall, this means that greater precipitation would drive δ^{13}C values down (more negative). There is also evidence that plant stomata opening is driven by soil water potential (Carminati and Javaux, 2020). Specifically, we think that formed C in plant inputs is lowered in δ^{13}C value, and is could be driving down δ^{13}C values in the soil profile. However, we lack isotopic measurements of plant tissue and think it would be a valuable avenue for future work. Soil δ^{13}C values in the Spring and Winter treatments were not significantly different from the Control, and the high variability in this measurement makes it hard to detect if we are seeing this pattern in our data, however there is some evidence of slightly lower δ^{13}C values in the spring and winter treatments from 0-100cm and again from 120-200cm (Fig. 2e).

Although plant communities are sensitive to changing precipitation regimes, microbes are resilient to precipitation shifts. Specifically, there is evidence that microbial community structure are resilient to long term shifts in precipitation seasonality, even if there are shifts in plant community structure (Cruz-Martínez et al., 2009). This capacity is likely because the climatic history of mediterranean ecosystems would select for microbial populations resilient to soil moisture fluctuations (Cruz-Martínez et al., 2009). While microbial communities are resilient to long term changes in moisture regime, there is evidence that they can respond rapidly to immediate changes in environmental conditions, which may be missed in long term studies (Cruz-Martínez et al., 2012). For example, subsoil microbial communities quickly respond to added C (Min et al., 2021) and old C can be quickly mineralized (Fontaine et al., 2007). These studies suggest that increases in C translocation to subsoils could stimulate the loss of ancient buried C through a potential priming effect, where additions of fresh C can enhance the decomposition of harder to decompose or mineral associating C (Keiluweit et al., 2015; Kuzyakov et al., 2000). While this study lacked explicit measurement of microbial communities with precipitation addition, we did observe increased translocation of C throughout the depth profile with precipitation addition. This ability of subsoil microbial communities to quickly take advantage of fresh C inputs could affect the long-term sequestration potential of subsoils affected by increased precipitation.

4.3 Implications for C sequestration potential of deep soils in grasslands

While our results suggest possible C accrual in subsoils with winter addition of precipitation, it is important to consider mechanisms for destabilization of subsoil C. Addition of fresh C to subsoils is identified as a potential destabilization mechanism due to priming effects (Rumpel and Kögel-Knabner, 2011). The addition of carbon (both microbial and plant derived) in subsoils in the winter treatment could fundamentally alter C cycling in subsoils. Greater work is still needed on what proportion of added C can become mineral associated in subsoils or is quickly mineralized by soil microbes. Current evidence suggests that fresh C is quickly mineralized at depth (Fontaine et al., 2007), but few studies have looked at fresh C partitioning to the mineral associated fraction in subsoils. This added C could also be affecting microbial community structure as well as increase the formation of necromass at depth. There is also evidence for changing porosity and soil structure to impact the structure of microbial communities in soils (Wilpiszeski et al., 2019). Overall, interactions between changing soil water conditions and C addition to subsoils is dependent on the seasonality of this added precipitation in a Mediterranean grassland, and could affect the sequestration potential of subsoils in grasslands under climate change.

5 Conclusion

This study leveraged a long term (20 years) precipitation manipulation experiment to investigate how changing precipitation amount and seasonality would affect soil C, N, and functional group chemistry in deep soils of a California grassland. We measured a suite of soil chemical characteristics, stable isotopes, C stocks, and performed Diffuse Reflectance Infrared Fourier transform Spectroscopy (DRIFTS) on all samples at 10cm increments for 0 to 3m and found greater cumulative C stocks in the winter treatment. Across all treatments, we found that soils from 1-3 m held nearly a third of the overall C stock. These results suggest that added precipitation over the winter in Mediterranean grasslands can alter plant inputs and enhance C stocks in deep soils, and highlights the importance of measuring soil C and functional group chemistry to greater depths. This study found that increased precipitation has the potential to increase C translocation to deeper layers, and that added winter precipitation causes a shift towards more abiotic control on soil C cycling. Changing precipitation amount and seasonality need to be taken into account when considering the C sequestration potential of Mediterranean grasslands.

Author Contributions

Conceptualization of the project led by AAB. Field and lab work was carried out by LMW. Formal analysis and investigation done by LMW with supervision and contributions from all authors. LMW prepared the manuscript with contributions from all co-authors.

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Competing Interests

The authors declare that they have no conflict of interest

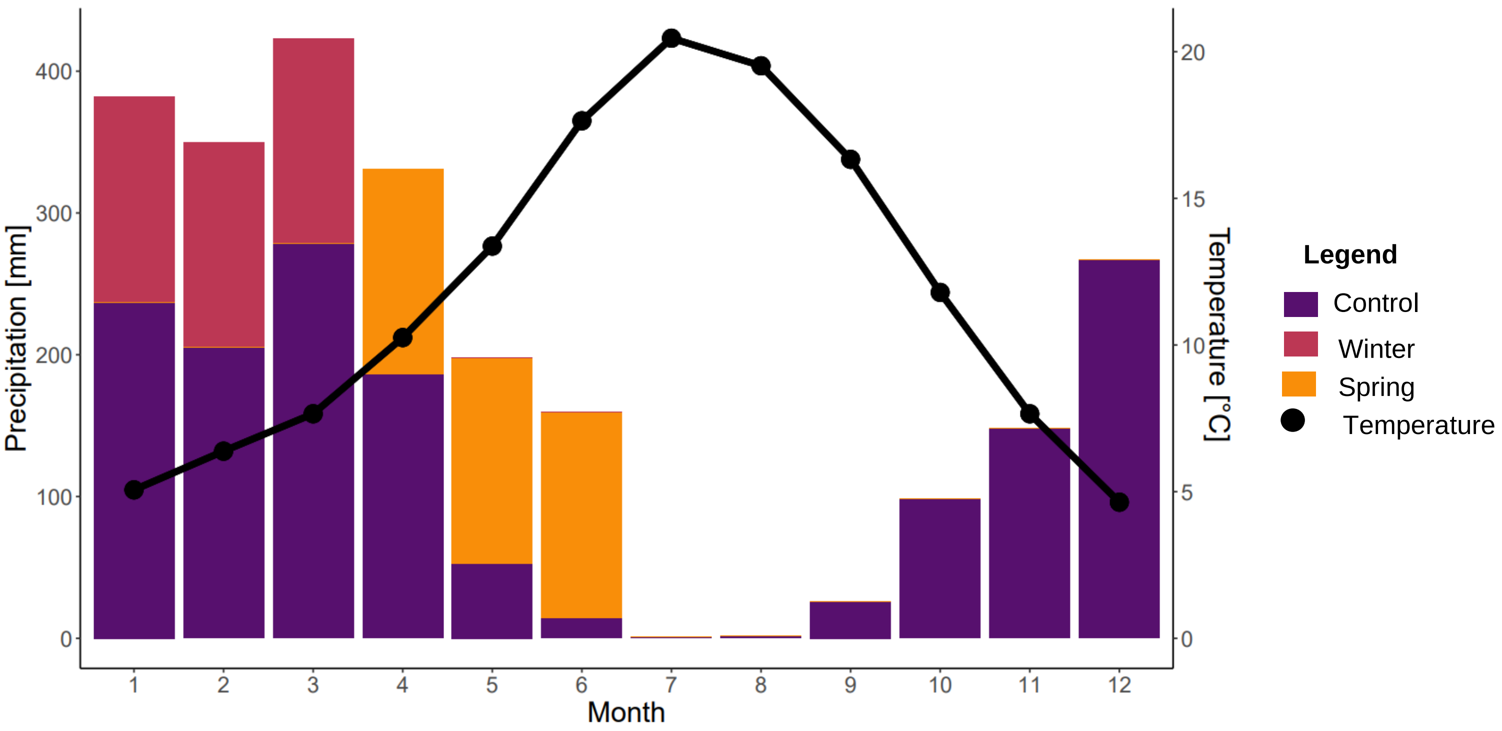

Figure 1. Precipitation and temperature over a year at Angelo Coast Range Reserve. The control indicates ambient precipitation, whereas the added precipitation for the winter and spring treatments is shown in the months that it is added. Data was sourced from Dendra (a cyber-infrastructure project for real time data storage) for Angelo from 2012-2022. Months are numbered

Table 1. Functional group assignments for the bands of interest used to evaluate DRIFT spectra (based on Mainka et al. 2022)

Functional Group	SOM type	Wavenumber center (range) cm^{-1}
Aliphatic C-H stretch	Simple Plant Matter	2925 (2976-2998) 2850 (2870-2839)
Aromatic C=C stretch	Complex Plant Matter	1525 (1550-1500)
Amide, quinone, ketone C=O stretch, aromatic C=C, and/or carboxylate C-O stretch	Microbially associated OM	1620 (1660-1580)

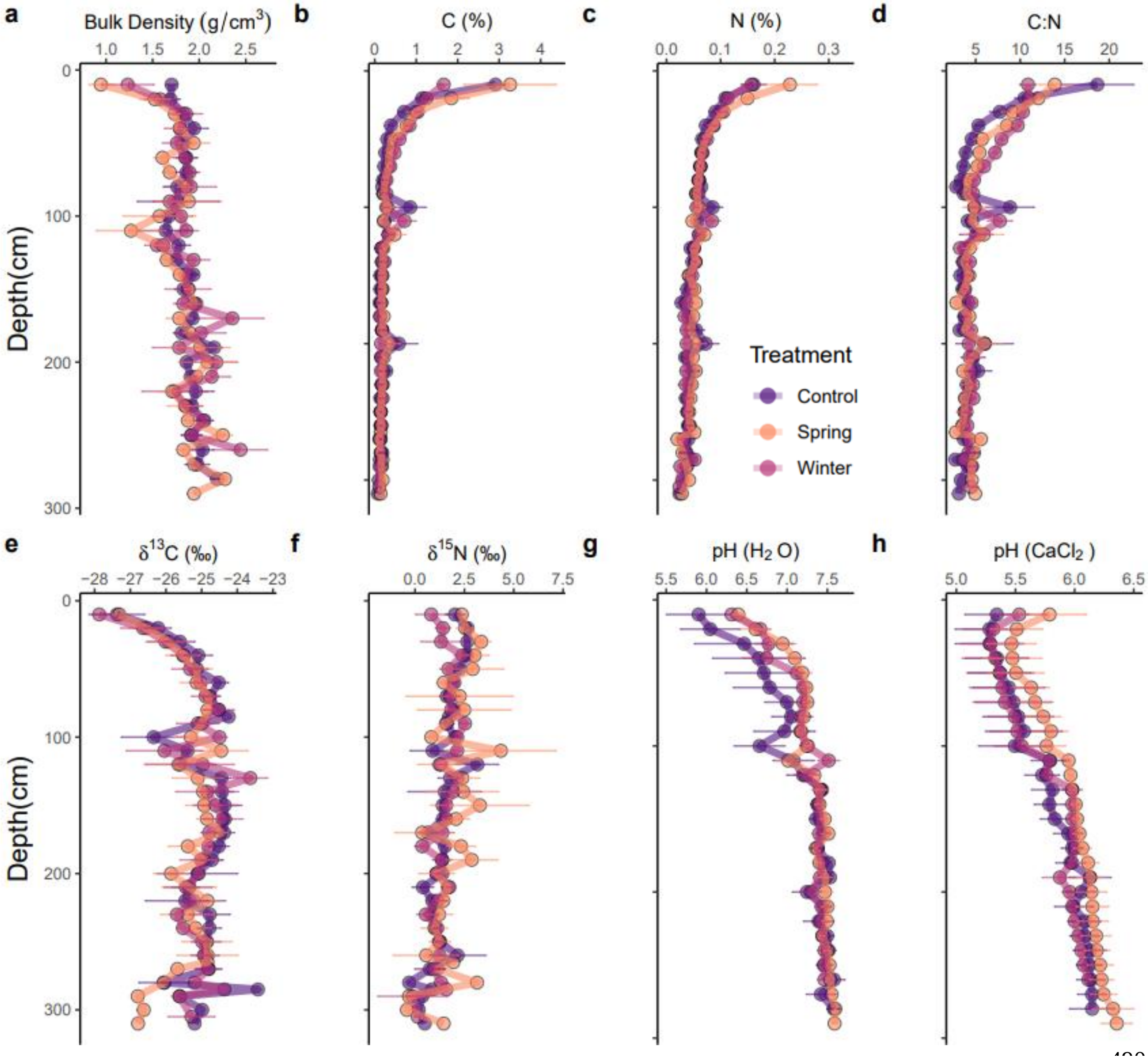

Figure 2 (a-h). Physical and chemical parameters for all treatments across the depth profile. All data are shown as means with standard error (n =3 for each treatment). Panel a shows Bulk density in g/cm^3 for all treatments, panels b and c show C(%) and N(%) respectively. Panel d shows C:N ratios. Panels e and f show δ^{13}C and δ^{15}N stable isotope values. Panels g and h show pH in H_2O and $CaCl_2$ respectively.

Table 2. Calculated cumulative carbon stocks for each treatment to examine how total carbon stocks might be changing with precipitation addition. The winter and control treatments had the greatest cumulative carbon stocks based on three cores of 0-300cm per treatment (n=9);standard error is shown in parentheses.

Treatment	**Cumulative carbon stock (0-300cm) (g/cm^2)**
Control	191.2 (36.7)
Winter	200.5 (34.5)
Spring	171.4 (13.7)

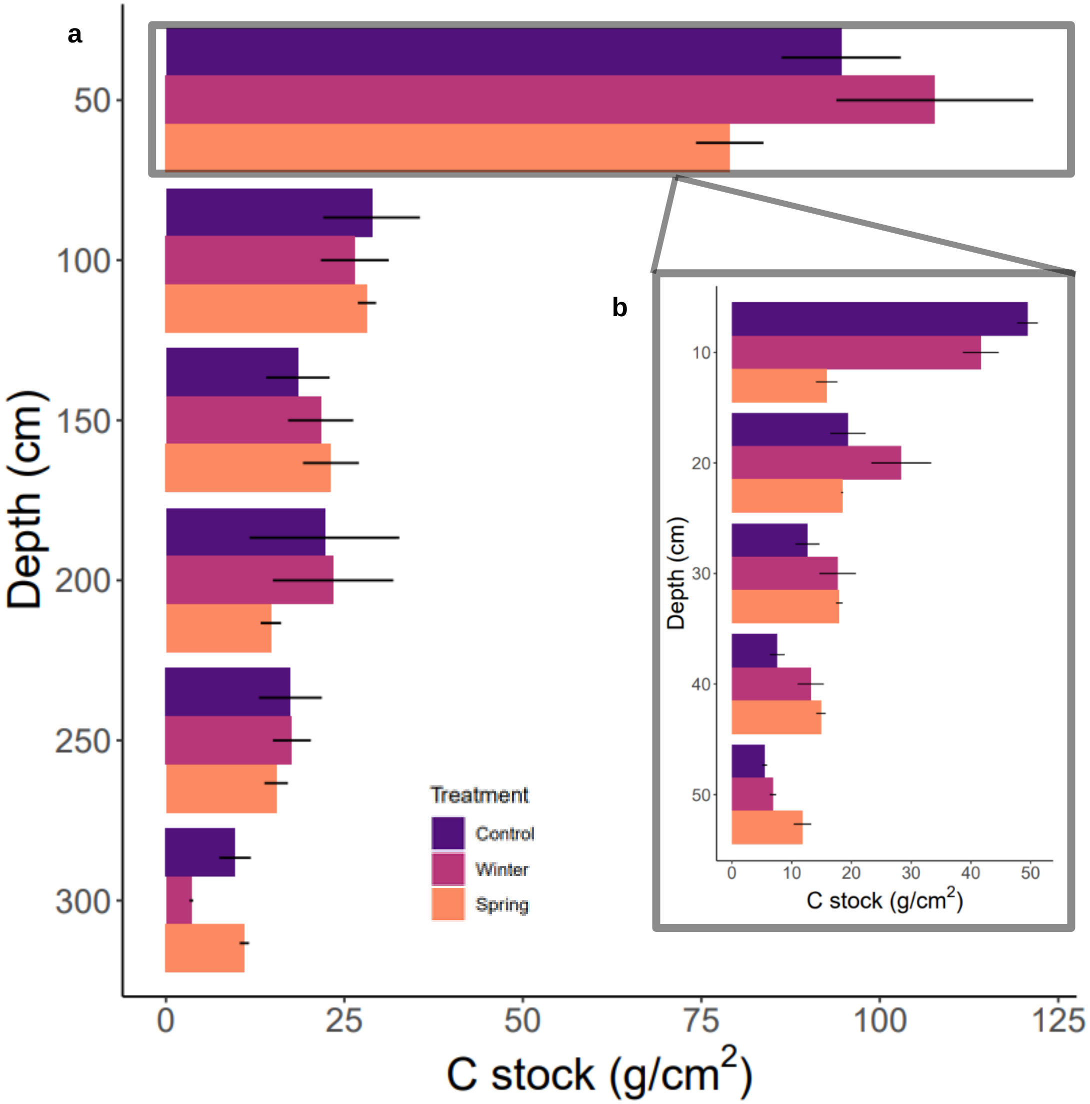

Figure 3. Carbon stocks throughout 0-300 cm cores across control treatment as well as winter and spring precipitation additions. The average calculated carbon stocks in A) 50 cm depth increments with standard error and B) inset includes higher resolution for the top 50cm with 10cm increments.

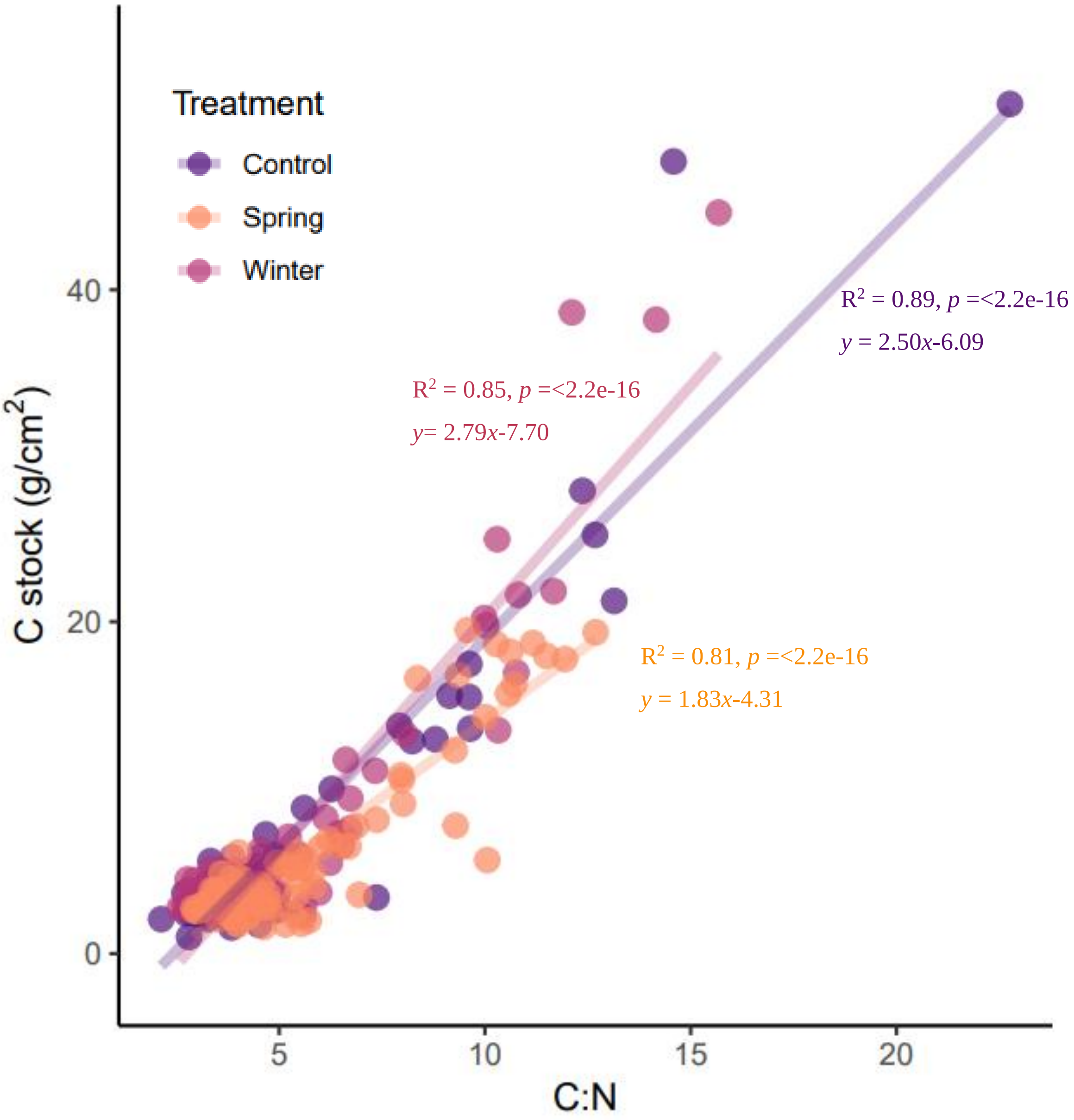

Figure 4. Linear regressions of C:N and C stock for all depths and treatments reveal differences in nutrient dynamics between spring treatments. All linear regressions are significant but the winter treatment slope is more shallow than the control and spring treatments, which have similar slopes and higher C:N at the surface.

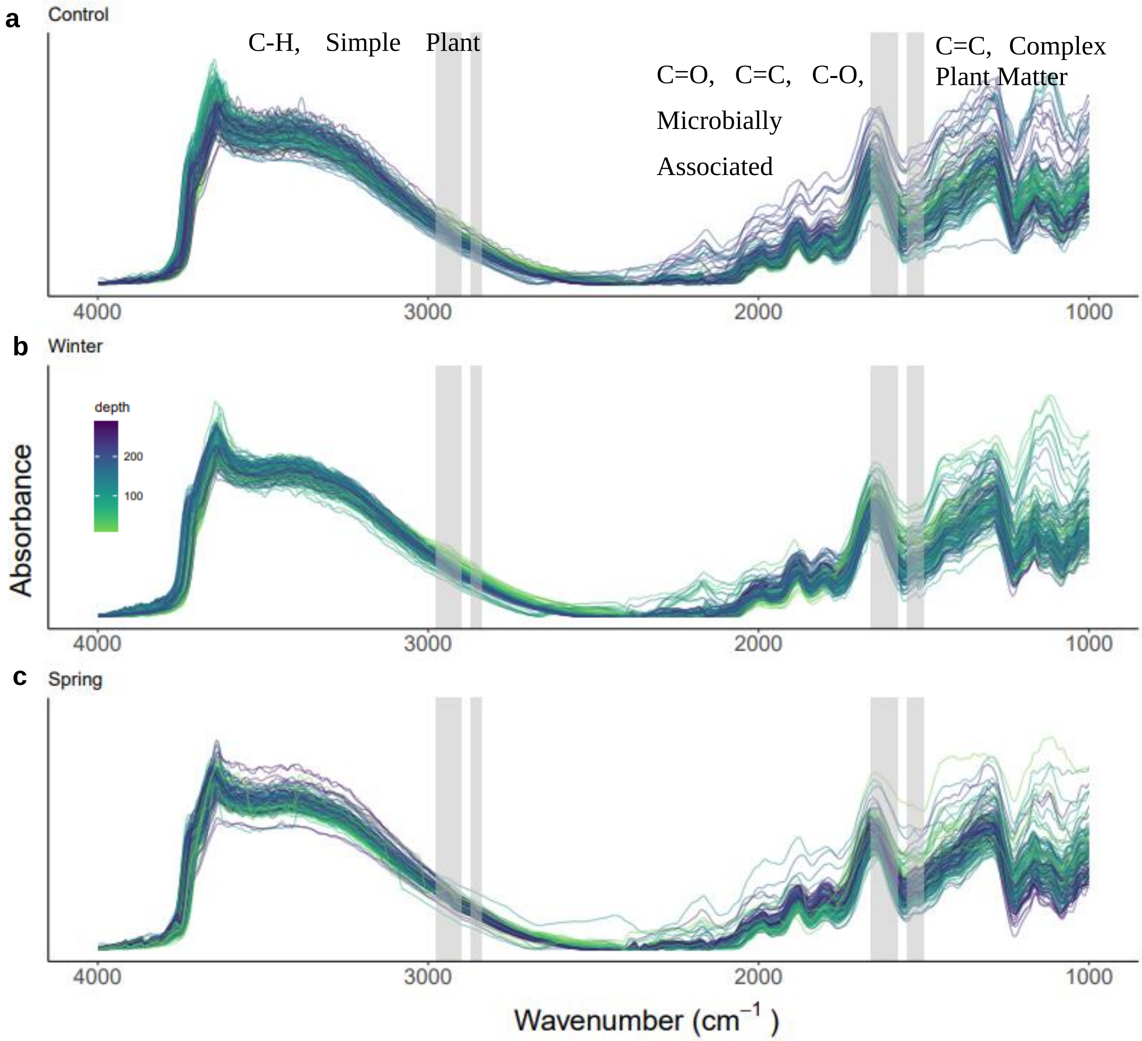

Figure 5 (a-c) . DRIFTS spectra across treatments and depths labelled with wavenumbers of interest: aliphatic compounds and simple plant matter (2976-2998 cm^{-1} and 2870-2839 cm^{-1}); aromatic compounds and complex plant matter (1550-1500 cm^{-1}); amide, quinone, ketone stretch, aromatic and/or carboxylate stretch, and microbially associated OM (1660-1580 cm^{-1}). The Control, winter, and spring treatments are shown in panels A, B, and C, respectively, and colors represent the depth gradient.

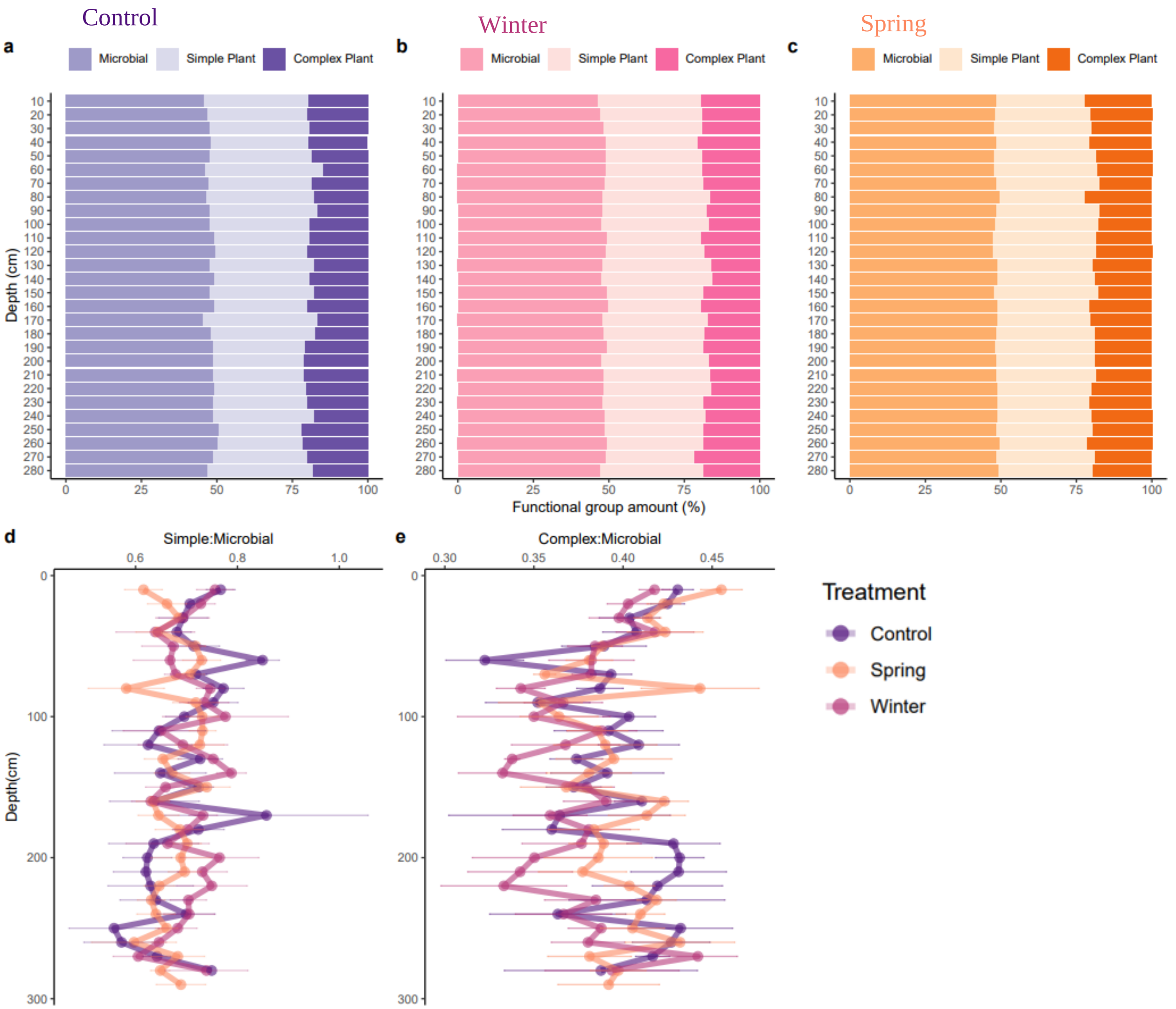

Figure 6 (a-e). Proportions of integrated area for areas of interest in DRIFTS data, which indicate the dominance of microbially associated OM across treatments and depths. Areas of interest on DRIFTS spectra include simple plant derived functional groups (2976-2998 cm^{-1} and 2870-2839 cm^{-1}), complex plant derived functional groups (1550-1500 cm^{-1}), and microbially associated OM (1660-1580 cm^{-}). The proportional area of interest for each 10cm depth interval for the A) control treatment, B) winter treatment, and C) spring treatments. The ratios of D) simple plant matter to microbial plant matter and E) complex plant matter to microbial plant matter by depth is also shown with averages and standard error for each 10 cm depth interval.

Table 3 Results from GAMM models for predicting $\delta^{15}N$ (a) and predicting SOC (b

	Model and terms	**Estimate**	**z value**	***P* value**	**AICc**	**Deviance Explained (%)**
a)	**Model 1: abiotic & biotic factors**					
	$\delta^{15}N$	1.56	29.35	<2e-16	742.4	19.9
	Depth (Control)			<2e-16 ***		
	Depth (Winter)			1.55e-06 ***		
	Depth (Spring)			2.98e-06 ***		
	simple plant: microbial (Control)			0.34		
	simple plant: microbial (Winter)			0.0076 **		
	simple plant: microbial (Spring)			0.31		
	complex plant: microbial (Control)			0.48		
	complex plant: microbial (Winter)			0.0023 **		
	complex plant: microbial (Spring)			0.019 **		
	Model 2: abiotic factors					
	$\delta^{15}N$	1.56	30.38	<2e-16	747.9	17.8
	Depth (Control)			<2e-16 ***		
	Depth (Winter)			4.04e-05 ***		
	Depth (Spring)			6.4e-04 ***		

b)	**Model 1: abiotic & biotic factors**					
	log (SOC)	-1.44	-26.3	<2e-16	151.9	63.4
	Depth (Control)			<2e-16***		
	Depth (Winter)			<2e-16***		
	Depth (Spring)			<2e-16***		
	simple plant: microbial (Control)			0.25		
	simple plant: microbial (Winter)			0.088		
	simple plant: microbial (Spring)			0.096		
	complex plant: microbial (Control)			0.078		
	complex plant: microbial (Winter)			0.13		
	complex plant: microbial (Spring)			0.69		
	Model 2: abiotic factors					
	log (SOC)	-1.46	-25.6	<2e-16***	153.9	61.7
	Depth (Control)			<2e-16***		
	Depth (Winter)			<2e-16***		
	Depth (Spring)			<2e-16***		

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