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The Influence of Compost on Rangeland Soil Microbial Community Composition and Soil

Organic Carbon Sequestration

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THE INFLUENCE OF COMPOST ON RANGELAND SOIL MICROBIAL COMMUNITY COMPOSITION AND SOIL ORGANIC CARBON SEQUESTRATION

by

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Thesis

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The Influence of Compost on Rangeland Soil Microbial Community Composition and Soil Organic Carbon Sequestration

Abstract

Atmospheric concentrations of CO_2 have reached unprecedented levels, posing a threat to the health of the biosphere. Soils store the largest amount of terrestrial carbon (C) and can be used as a method for sequestering atmospheric C. The cycling of C between the atmosphere and terrestrial ecosystems is directly influenced by soil microbial activity and respiration. Compost amendments to soil systems have the potential to shift the composition of the microbial community and to increase C sequestration potential. Rangeland ecosystems represent a valuable opportunity for implementing climate mitigation practices with compost amendments. This study aims to answer the following questions: (1) Does a one-time compost addition to rangeland soils induce a shift in the microbial community one year post application? (2) Does compost addition increase or decrease the rate of CO_2 efflux over the growing season? and (3) Does MRT of SOC differ between soils treated with compost and soils not treated with compost?

Three rangeland sites located in Gunnison, Colorado were manipulated with compost additions in June of 2019: Coldharbour (CH), Parker Pastures (PP) and Wiley Lane (WL). Soil moisture, temperature and respiration rates were measured throughout the 2020 growing season. Soil samples were collected and analyzed for bulk density, SOC pool, nitrogen (N) pool and microbial phospholipid fatty acids. A one-time compost application had no effect on the microbial community composition of rangeland soils one year post application. Daily CO_2 efflux rates were not influenced by compost application, however, the cumulative release of C-CO_2

over the growing season was higher in the non-composted soils. The largest difference of cumulative efflux between treatments was seen at the CH site where control soils had an average $C-CO_2$ efflux of 811.1 g C $m_2^{-1}d^{-1}$ and treatment soils had an average $C-CO_2$ efflux of 652.8 g C $m_2^{-1}d^{-1}$. Mean Residence Time (MRT) of SOC across sites ranged from 0.64 – 3.21 years and was significantly higher in the treatment soils than in the control soils. On average, compost application treatment increased MRT of SOC by 0.35 years across sites. As the C pools were not significantly different between treatments, the significantly lower efflux observed in the treatment soils was responsible for the increase in MRT of SOC found. These findings present a valuable representation of how improved land management practices on rangeland soils can positively contribute to soil C sequestration.

Introduction

Soil carbon sequestration and mean residence time

Elevated CO_2 in the atmosphere has caused global temperatures to rise and ecosystem dynamics to shift in a detrimental manner. Sequestering carbon (C) in soils may be a beneficial method for reducing atmospheric CO_2 concentrations and mitigating the negative impacts of climate change (Ryals & Silver, 2013). The most important aspect of increasing soil C sequestration potential involves increasing the mean residence time (MRT) of C within the soil profile. In soil biology, MRT is defined as the duration that a compound persists in the soil profile. Soil microbial communities influence this residence time through the release of CO_2 as a by-product of their respiration, but also through their ability to transform organic C into stable aggregates and to move C deeper into the soil profile, extending the MRT of soil organic carbon (SOC) (Chen 2013; Rabbi et al., 2013). Soils generally have the potential to store significant amounts of organic C, however, many soils have been degraded as a result of anthropogenic activities (Lal, 2007). These degraded, nutrient-poor, soils are not able to store C as long or as effectively as healthy, nutrient-rich, soils (Zhang et al., 2019). It may be possible, however, to restore soils that have been degraded to increase C sequestration abilities and improve soil health through the addition of compost.

Compost amendment to rangeland soils

Rangeland ecosystems present a valuable opportunity for enhancing C sequestration as nearly one-third of global terrestrial C is stored in the vegetation and soils of rangelands (Billings et al., 2000). Without proper management, rates of C sequestration decline over time. In the United States, rangelands with proper management have the potential to sequester 12 million tons of C

per year (Morgan et al., 2010). In relation to global studies, total CO_2 emissions in the year 2020 resulting from anthropogenic activities such as burning fossil fuels, deforestation and land use change were approximately 40 billion tons (Global Carbon Budget, 2020). While this number is an improvement in comparison to the previous year, when 43 billion tons of CO_2 were released to the atmosphere, it is clear that methods for reducing these concentrations are of paramount importance. Methods such as adjusting stocking rates, increasing plant biodiversity and adding compost have the potential to increase the rate of C sequestration in improved rangelands (Morgan et al., 2010).

The application of compost to similar ecosystems, such as annual grasslands, has shown to significantly increase C storage (Ryals and Silver, 2013; Villa and Ryals, 2021). In a meta-analysis conducted by Gravuer et al. (2019), increased concentrations of SOC were detectable after two years on rangeland sites that received single applications of compost. The length of time that a single compost application may impact a site is influenced by the concentration of total nitrogen (N). An amendment with a high N concentration will more strongly increase plant productivity, thus supporting a continuation of C inputs to the soil (Gravuer et al., 2019). In dryland ecosystems, however, observed initial increases in above ground net primary productivity following high N compost additions were eventually found to decrease over the long term (Gravuer et al., 2019).

Microbial activity & C cycling interactions

Compost amendments and increased nutrient availability in soils have both direct and indirect effects on soil health and C cycling. Directly, compost amendments can increase the amount of available soil nutrients, particularly N and labile C, a form of SOC that is easily and readily digested by most soil microbes. Microbial activity stimulation increases the processing of labile

C and the release of CO_2 to the atmosphere (Bastida et al., 2008). As labile C has the most rapid turnover time, the oxidation of labile C is the main driver of the flux of CO_2 between soil and the atmosphere. Indirectly, compost amendments influence the flux of SOC and atmospheric CO_2 via changes in primary productivity of vegetation. Increased nutrient availability promotes plant productivity, and thus increased atmospheric CO_2 uptake and the eventual return of C to the soil in the form of dead plant biomass (Morgan et al., 2010; Figure 1). As organic matter in the form of dead plant biomass accumulates, increased microbial C cycling and C storage can generate a positive feedback loop, further enhancing productivity and C sequestration (Jastrow et al., 2007). However, increased plant productivity can also lead to an increase in root biomass and consequently root respiration, adding to the overall output of CO_2 to the atmosphere from soil respiration. This flux between soil and atmosphere can be viewed as an exchange of C inputs and C outputs, whereby C sequestration occurs when C inputs to the soil exceed the C outputs to the atmosphere.

Microbial community structure influences function

Variation in the relative contribution of each soil microbial group that performs different C cycling functions impacts the amount of SOC that is released and stored. To analyze and identify soil microbial communities, the phospholipid fatty acid (PLFA) analysis method has been widely used. In fact, long term C storage was found to be associated with increased total PLFA biomass following an organic manure compost amendment in agricultural croplands (Kong et al., 2011). Yet the specific influence on SOC is different depending on the type of soil microbe. In general, fungi and bacteria are the main contributors to the cycling of C in soils. When nutrients are abundant, fungi form dense networks of hyphae, the filaments responsible for penetration into substrates and translocation of chemical compounds. Bacteria in soil microbial communities are

classified as either gram-positive (GP) or gram-negative (GN). GP bacteria contain more peptidoglycans in their cell walls, which has been suggested to positively influence the accumulation of decay resistant C, while GN bacteria generally decompose a variety of organic C sources (Hill et al., 2008). Actinomycetes are a specific group of GP bacteria that are known to decompose old soil organic C and exist in low nutrient soils (Zhang et al., 2012). For this reason, the distribution of microbial communities generally involves higher concentrations of actinomycetes and GP bacteria at lower soil depths, with GN bacteria and fungi having higher concentrations near the surface where there is usually higher SOC concentrations (Fierer et al., 2003).

It has been widely found that a site's C sequestration potential is correlated with its ratio of fungi to bacteria, whereby a greater fungal:bacterial dominance is associated with greater C sequestration potential (Jastrow et al., 2007; Bailey et al., 2002; Zhang et al., 2019). Moreover, the ratio of GP:GN bacteria has been used as an indicator of the relative C availability for soil microbial communities due to their different associations with different C forms (Fanin et al., 2019). In high fertility soils, increases in abundances of GP bacteria, fungi and anaerobes accompanied by decreases in GN bacteria, actinomycetes and aerobes was associated with the observed increase in C storage (Zhang et al., 2019). These shifts in nutrient availability and microbial community composition have the potential to increase soil C sequestration, however, larger applications of compost can also lead to higher amounts of CO_2 released via microbial respiration (Torres et al., 2015; Bastida et al., 2008; Bastida et al., 2013). Gude et al. (2012) found that increased C inputs to a degraded agricultural system stimulated an increased rate of C turnover resulting in less soil C than soils without C additions, a concept known as the priming effect. These confounding results highlight the need for a better understanding of the influence of

soil microbial communities on the storage and loss of C in degraded rangeland soils subject to compost additions.

This study aims to answer the following questions: (1) Does a one-time compost addition to rangeland soils induce a shift in the microbial community one year post application? (2) Does compost addition increase or decrease the rate of CO_2 efflux over the growing season? and (3) Does MRT of SOC differ between soils treated with compost and soils not treated with compost? Utilizing PLFA analysis, measurements of soil respiration and soil C content, our goal is to provide insight into the influence of compost additions on the C sequestration potential of rangeland soils thereby influencing land management strategies in support of climate change mitigation.

Methods

Study Area

All sites are located in the Gunnison Valley of Colorado, USA (Fig. 2). This region is characterized as a sagebrush-steppe ecosystem existing at an average elevation of 2,347 m in a hardiness zone of 4a (https://www.plantmaps.com). Mean annual high and low temperatures are 12°C and -6°C, respectively. The mean annual precipitation is 27 cm and annual snowfall is 114 cm (https://www.usclimatedata.com). The research sites are classified as perennial grasslands. The 60-day growing season begins in late May and ends in late July. This time period is classified as 'frost-free' days, however the cool-season grasses dominant in these systems are

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most productive between mid-April and late-August. Data collection occurred from June 28, 2020 to September 23, 2020, referred to as the 'growing season' throughout the paper.

The perennial grassland plant communities are dominated by the perennial grasses: Meadow Foxtail (*Alopercus pratensis),* Kentucky Bluegrass *(Poa pratensis)* and Timothy *(Phleum pretense)*; as well as the perennial rush: Baltic Rush (Juncus balticus) and the perennial forbs: Alsike Clover *(Trifolium hybridum),* Mammoth Clover *(Trifolium hybridum L)* and Dandelion *(Taraxacum officinale)*(Cooper et al., 2021)*.* Invasive species existing at these sites include: Prostrate Knotweed (*Polygonum aviculare*), Canada Thistle (*Cirsium arvens*), and Curly Dock (*Rumex crispus*) (Cooper et al., 2021). The rangeland soils consist of well-drained, gravelly loam residuum weathered from granite (http://websoilsurvey.sc.egov.usda.gov).

In June of 2019, three rangeland sites were selected to conduct our compost addition experiment: Coldharbour Ranch (CH) (Lat. 38.52098, Long. -106.78583), Parker Pastures (PP) (Lat. 38.56311, Long. -106.93822) and Wiley Lane Ranch (WL) (Lat. 38.62508, Long. -106.95143) (Figure 2). Grazing regimes and land management techniques vary across sites. The CH Ranch research site is a sector within Coldharbour Institute, a learning laboratory that demonstrates regenerative ranching practices. Both PP and WL Ranch sites are operated by private ranchers. All sites are located within 15 km of each other and received varying degrees of irrigation and grazing during the months of June, July, August and September.

Compost amendment

Each site contained five replicate plots per treatment (control and compost addition), with each plot measuring 5 m x 10 m. A single application of a class A biosolid compost, Gunny Gold, was applied to each site at a rate of 5 cm per treatment plot between the dates of June 17 and June 21,

2019 (Cooper et al., 2021). This compost is a product of the City of Gunnison's wastewater treatment plant and is comprised of municipal sludge and industrial wood products waste resulting in a nitrogen and C rich blend, with a C:N ratio of 14:1 (https://www.gunnisonco.gov/departments/public_works/wastewater_treatment_plant/gunnison_gold_compost.php; Cooper et al., 2021). The amount of compost applied per plot was 23.4 m^3, and per site was 117 m^3.

Soil collection

Soils were collected to a 10 cm depth using a 5 cm diameter soil corer over the period of August 30th – September 5th, 2020. At each plot, three cores were collected and separated by their organic and mineral layers and then pooled by layer resulting in one soil sample per soil layer for each plot. Bagged samples were stored in a cooler with ice packs during transportation to the laboratory, where they were then placed in a refrigerator at 4°C until further processed. A total of 44 soil samples were collected from all three sites in accordance with the presence of mineral and/or organic layers in each plot. A mineral soil sample from a PP control plot was removed from analysis due to contamination during preparation resulting in a total of 43 soil samples used in analysis.

Preparation of soil samples for further laboratory analysis began within 24 hours after soil collection. Live plant material was removed and all soils were sieved to 2 mm. Coarse fractions were dried at 65°C for 48 hours and then weighed. Rocks were weighed and their volume quantified using water displacement. Post homogenized soil weights were taken and samples were divided into various volumes for further analysis. To determine soil moisture, soils were dried at 105°C for 48 hours and weighed. Dry and wet soil weight values were used to calculate % soil moisture (Equation 1). The oven dry equivalent (ode) sieved bulk soil weight used in the

calculation of bulk density (Bd) was derived from subtracting the rock volume from the post-homogenized wet soil weight and then multiplied by the percent soil (dry soil weight/wet soil weight). The ode sieved bulk soil weight was used to calculate Bd in relation to the volume of soil core collected (Equation 2).

(Equation 1) $\% \: soil \: moisture \: (g) = 100 * (wet \: soil \: wt.(g) - dry \: soil \: wt.(g)) \div dry \: soil \: wt.(g)$

(Equation 2) $Bd \left(\frac{g}{cm^3}\right) = ode \: sieved \: bulk \: soil \: wt.(g) \div core \: volume \: (cm^3)$

Microbial Community Characterization

Soils for the phospholipid fatty acid method were air-dried at room temperature for a period of two weeks. Samples were analyzed for microbial biomass using the phospholipid fatty acid method by Ward Laboratories. Their protocols involve the use of extracting solvents and a centrifuge to allow for the separation of the organic fraction containing the fatty acids. The desired fraction of phospholipid fatty acids are then methylated and analyzed on a gas chromatograph using Agilent's ChemStation and MIDI's Sherlock software (Ward Laboratories SOP FAME & PLFA in Soil, 2020) (Ward Laboratories, Inc., Kearny, NE).

PLFA analytical tests reports several functional groups: total bacteria, total fungi, and protozoa. Within total bacteria, values for gram-positive bacteria and gram-negative bacteria concentrations are included. Total gram-positive bacterial concentrations are inclusive of the functional group actinomycetes, while total gram-negative bacterial concentrations are inclusive of the functional group rhizobia. The PLFA biomass of total fungi is reported as the sum of

saprophyte and arbuscular mycorrhizae concentrations. Values for undifferentiated PLFAs are also reported yet were not included in this analysis.

Duplicate samples from the following four plots and horizons were included to provide some estimate of within-plot variance: CHC1-Org, WLT1-Min, WLC1-Min, PPC2-Min. For these particular samples, all PLFA biomass and ratio values reported and analyzed herein represent averages of the duplicate samples.

Soil respiration

Soil respiration was measured in the SE corner of each treatment plot and in the NE corner of each control plot (Figure 3), using PVC soil collars measuring 10 cm in diameter and 4.4 cm in depth and were inserted approximately 3 cm deep into the soil profile. Vegetation was clipped and removed from the area within the soil collars before each measurement. Respiration measurements were taken using a LI-COR 6400-09 (LI-COR, Inc., Lincoln, NE, USA) fitted with a soil chamber attachment. On a bi-weekly basis, the soil chamber was placed on the soil collars and set to measure efflux over 3 cycles per plot. Soil respiration measurements at the Coldharbour site began later than the other two sites due to equipment malfunction.

Soil moisture and temperature

Soil volumetric water content (VWC) was collected in each plot in conjunction with soil respiration measurements using a HydroSense II Soil Moisture Probe (Campbell Scientific, Inc., Logan, UT, USA). VWC is averaged over the length of the measurement rods, providing a representation of the soil moisture that is present to a depth of 20 cm. Three measurements of VWC were taken per plot on a bi-weekly basis over the period of June 28, 2020 – September 23, 2020. Three replicates within a plot were averaged resulting in one VWC value per plot per time

point. Values obtained at WL on June 28, 2020 include measurements taken from three treatment plots and no control plots due to human error. Values for June 3, 2020 at PP are missing due to equipment breakage.

Soil temperature measurements at each plot were collected continually over the growing season using iButton Temperature Data Loggers (iButtonLink LLC, Whitewater, WI, USA). The loggers were placed in each plot at approximately 3 cm under the soil surface on June 20, 2020 and set to log temperature data every three hours until August 26, 2020. Two iButtons from the PP site could not be recovered, therefore only four control and four treatment plots were measured at this site.

Total soil C and N

Soils were dried at 65°C for 48 hours then ground using a mortar and pestle. Ground samples were placed in 20-mL scintillation vials and shipped to the University of California Merced and processed in their Stable Isotope Ecosystem Laboratory. A Costech 4010 Elemental Analyzer was used to analyze the amount of C and N in all soil samples (Costech Analytical Technologies Inc., Valencia, CA, USA). This instrument begins by combusting the soil sample to produce gases that are then separated using a gas chromatograph. The CO_2 and N gases then pass through an isotope ratio mass spectrometer, after which 50 µg C and 50 µg N are packed into tin capsules and analyzed in relation to reference samples with known C and N amounts (isotopes.ucmerced.edu).

The amount of SOC was originally reported in %C , these values were converted using (Equation 3) to obtain the proper units utilized in the calculation of MRT. This equation was also used to convert %N to total N pool. Original flux measurements were reported in μmols CO_2 m_2^{-1} s^{-1},

and converted using (Equation 4) to obtain the proportion of C contained in the CO_2 molecules released (C-CO_2). The total efflux in this equation was obtained by summing the daily efflux values over the entire growing season. MRT of SOC was calculated by dividing the carbon pool by the carbon flux (Equation 5).

Percent C and N values for the organic layers of plots PPT2 and PPT4 were missing due to equipment malfunction of the elemental analyzer. To maintain an equal number of replicates across sites and treatments, the average % C and % N values of the other three treatment organic layers were used in C pool calculations. This method was also used for the organic horizons at WLT2 and WLT4 as there was not enough sample collected from these plots to send for analysis. Original values for the N pool were not normally distributed, therefore log transformed values were used in analysis.

To calculate total C efflux over the entire growing season, the average respiration measurements obtained at each plot were used to estimate the daily respiration values for the days in between sampling days that were not sampled. This extrapolation method may possibly be over- or under-estimating the true daily respiration values, as one measurement could be used to represent daily values of up to 4 weeks at the WL and PP sites. At the CH site in particular, the respiration measurements obtained on July 17, 2020 were used to represent the daily respiration values for all days between June 21, 2020 and July 31, 2020. Because respiration measurements began and ended on different days at each site, this extrapolation method was used to begin measurements on June 21, 2020 and end measurements on September 30, 2020 for all sites. This ensures that an equivalent number of growing season days were used in the comparison of total efflux per treatment and site. As one control plot at CH was continuously inundated with water in the early growing season, respiration measurements began one month after all other plots at this site.

When calculating the total growing season efflux, an average of the total efflux found at the

other four control plots were used to represent this one control plot with missing measurements.

(Equation 3) $$SOC\ pool\left(\frac{gC}{m^2}\right) = soil\ depth\ (cm) \times Bd\left(\frac{gC}{cm^3}\right) \times \%C \times 10,000\left(\frac{cm^2}{m^2}\right)$$

(Equation 4) $$C-CO^2\ flux\left(\frac{gC}{m^2 day}\right) = total\ efflux\left(\frac{umolsCO^2}{m^2 sec}\right) \times \left(12\ (gC) \div 1*10^6(umols\ CO^2)\right) \times 86,400\left(\frac{sec}{day}\right)$$

(Equation 5) $$MRT\ (years) = SOC\ pool\left(\frac{gC}{m^2}\right) \div C-CO_2\ flux\left(\frac{gC}{m^2 day}\right) \div 365\left(\frac{days}{year}\right)$$

Data Analysis

Repeated measures three-way Analysis of Variance (ANOVA) was used to estimate the

influence of date, site and treatment on soil respiration and soil moisture. Respiration values

were not normally distributed, therefore statistical analyses were performed on the log

transformed values while non-transformed respiration rates were used in all figures. Two-way

ANOVAs were carried out to analyze PLFA biomass of individual functional groups, PLFA

ratios, bulk density, C and N pools, and C:N values; with site, treatment and the site*treatment

interaction as the main factors. Post Hoc analyses were carried out using Tukey's HSD test.

Non-linear regression was used to model relationships between soil respiration, soil moisture and

soil temperature. Models were fitted with 2nd degree polynomials as none of the relationships

exhibited a strong linear correlation. Temperature data was logged every two hours on a

continual basis; the values used in regression analyses were an average of the 24 – hour period

that coincided with the date that the soil respiration and soil moisture measurements were taken.

All statistical analyses were carried out using the RStudio program, version 1.2.5019 (© 2009 –

2019 RStudio, Inc.).

Results

Microbial Community Composition

Across sites total PLFA biomass values ranged from 639.93 ng/g – 20,861.26 ng/g. Total

biomass did not differ significantly between treatments (Figure 4). Total bacterial biomass did

not differ between mineral and organic horizons, nor between control and treatment samples; yet

there was a general trend observed for an increase in total bacterial biomass with treatment ($F =$

$1.4_{1, 32}$, $p = 0.236$). Total fungal biomass did not differ between mineral and organic horizons,

nor between control and treatment samples (Table 1).

Further analysis of fungal communities showed no effect on concentrations of either saprophyte

or arbuscular mycorrhizae communities in any site or treatment. Yet a general trend was

observed for an increase in the biomass of arbuscular mycorrhizae with treatment ($F = 1.4_{1, 32}$, p

$= 0.243$). Similarly, no effect was found on the individual bacterial communities of total GP,

total GN, actinomycetes, or rhizobia. Yet there was a general trend observed for an increase in

total GP and total GN bacterial communities with treatment (GP bacteria: $F = 1.5_{1, 32}$, $p = 0.227$;

GN bacteria: $F = 1.4_{1, 32}$, $p = 0.244$). Additionally, no effect of treatment was observed on the

biomass of protozoan communities, yet a general trend was observed for an increase in biomass

with treatment ($F = 1.2_{1, 32}$, $p = 0.268$).

F:B ratios ranged from 0.1095 ng/g – 0.3392 ng/g, yet no significant difference was found

between treatments. However, F:B ratios did significantly differ between the three sites, with the

lowest value occurring at PP and the highest value occurring at CH ($F = 4.3_{2, 32}$, $p = 0.023$). No

interaction between site and treatment was observed. GP:GN ratios ranged from 0.4181ng/g –

2.234 ng/g, yet no significant difference was found between sites or treatments.

Soil Respiration

Average daily respiration values differed across sites ($F = 61.5_{2, 156}$, $p < 0.0001$) and with time (F

$= 154.6_{1, 156}$, $p < 0.0001$), with the PP site exhibiting the highest rates of efflux in the early

summer, and the CH and WL sites having generally lower rates of efflux throughout the entire

growing season. No effect of treatment on average respiration rates was found at any of the sites.

However, there was a general trend for treatment to decrease respiration across all sites ($F = 2.7_{1,}$

$_{156}$, $p = 0.099$), with the CH site exhibiting this trend more so than the PP or WL sites (Figure 5).

We found a significant interaction between site and treatment ($F = 2.5_{2, 156}$, $p = 0.085$). At the PP

and WL sites, control plots had generally higher respiration rates in the early and mid-summer

with lower respiration rates in the late summer. An opposite effect was seen at the CH site where

treatment plots had higher respiration rates in the mid-summer and control plots had higher

respirations rates in the late summer. Between late June and early September, at the CH site,

peak respiration occurred on August 1, 2020, July 3, 2020, and June 28 at the CH, PP and WL

sites, respectively. Minimum respiration rates occurred on September 23, 2020 at the CH site,

September 20, 2020 at the PP site, and September 5, 2020 at the WL site.

Treatment had a significant effect on the cumulative C-CO_2 efflux over the growing season ($F =$

$4.7_{1, 24}$; $p = 0.0398$). At all three sites the control soils had higher C-CO_2 efflux over the growing

season than the treatment soils. The largest difference between treatments was seen at the CH site where control soils had an average C-CO$_2$ efflux of 811.1 g C m$_2^{-1}$d^{-1} ($\pm$ 39.0) and treatment soils had an average C-CO$_2$ efflux of 652.8 g C m$_2^{-1}$d^{-1} ($\pm$ 53.4). Overall C-CO$_2$ flux was largest for the PP site with an average value of 1,159.7 g C m$_2^{-1}$d^{-1} ($\pm$ 75.6) and lowest for the WL site with an average value of 502.7 g C m$_2^{-1}$d^{-1} ($\pm$ 33.8).

Soil Moisture & Temperature

Across all sites, VWC fluctuated between 4% and 58% across the months of June through September with peak values occurring on July 7, 2020 at PP, August 1, 2020 at CH and September 20, 2020 at WL. Minimum VWC values occurred on August 18, 2020 at PP, August 28, 2020 at CH and September 5, 2020 at WL (Figure 5). An effect of compost amendments on soil moisture was found at the CH site with the control soils having consistently higher VWC values than treatment soils (p-adj < 0.0001). No effect was found at either PP or WL sites.

Over the months of June through August minimum and maximum values of soil temperatures observed at CH were 4.0°C and 38.0°C; at PP were 1.5°C and 59.5°C; and at WL were –2°C and 66.5°C. Significant correlations were found between the variables of soil respiration, soil moisture and soil temperature when all sites, treatments and plots were included. Soil respiration was positively correlated with soil moisture but negatively correlated with soil temperature. Soil moisture and soil temperature were negatively correlated (Figure 6).

Bulk Density

Bulk density values ranged from 0.29 – 0.93 g cm^{-3} and significantly differed across sites and between treatments (Site: F = 19.57$_{2, 24}$; p < 0.001; Treatment: F = 6.54$_{1, 24}$; p = 0.017). There was no significant interaction between site and treatment. At PP and WL sites, average bulk

density values were lower in the treatment soils than in the control soils, yet at the CH site, the control and treatment soils had similar average bulk density values (Table 2).

Soil C and N

Soil C pools ranged from 288.4 – 752.94 kg C m_2^{-1} and soil N pools ranged from 23.57 – 62.74 kg N m_2^{-1}. C and N pools did not significantly differ between treatments yet both pools differed between sites (C: $F = 6.18_{2, 24}$; $p = 0.007$; N: $F = 9.93_{2, 24}$; $p = 0.0007$). Percent soil C ranged from 4.1 – 20.4% and percent soil N ranged from 0.32 – 1.67% and did not differ between treatment or across sites. C:N ratios ranged from 11.1 – 14.5 and significantly differed between treatment ($F = 6.92_{1, 24}$; $p = 0.015$). The CH and PP sites had higher average C:N ratios in the control soils than in the treatment soils. In contrast, at the WL site, the opposite effect is observed whereby the average C:N ratios of the treatment soils were higher than the control soils (Table 2).

A strongly positive relationship between C pool size and C-CO_2 efflux was observed in the treatment soils at all sites. No significant relationship was observed between C pool size and C-CO_2 efflux in the control soils (Figure 7). A larger range in C:N ratios was observed in the control soils, yet no significant relationships were observed between ratios and C-CO_2 efflux or MRT. However, in the treatment soils a significant negative relationship was observed between C:N ratio and C-CO_2 efflux; and a significant positive relationship was observed between C:N ratio and MRT (Figure 8).

Mean Residence Time

Mean Residence Time (MRT) of SOC across sites ranged from 0.64 – 3.21 years. Average MRT was 1.88 ($\pm$ 0.16) years in treated plots and 1.53 ($\pm$ 1.03) years in control plots (Figure 9; F =

$4.8_{1, 24}$; p = 0.0382). Across sites, the WL treatment soils had the highest average MRT of SOC at 2.23 (± 0.17) years, while PP control soils had the lowest average MRT of SOC at 1.04 (± 0.16) years. On average, compost addition treatment increased MRT by 0.35 (± 0.04) years across sites.

Discussion

This study sought to investigate the role of compost addition on the soil microbial community composition and the carbon sequestration potential of rangeland soils in a high elevation, arid region with a short growing season. Specifically, it sought to answer three main questions: (1) Does a one-time compost addition to rangeland soils induce a shift in the microbial community one year post application? (2) Does compost addition increase or decrease the rate of CO_2 efflux over the growing season? and (3) Does MRT of SOC differ between soils treated with compost and soils not treated with compost?

Compost had no effect on the composition of the microbial community. Yet fungal to bacterial ratios differed across sites, as well as soil moisture and soil respiration, highlighting the high degree of site variability found in this study. Soil moisture had a stronger influence on soil respiration than did soil temperature. While the average daily rates of CO_2 efflux were unaffected by compost addition, the control soils at all sites showed higher cumulative C-CO_2 efflux over the growing season. While the C and N pools did not differ between treatments, the C:N ratios were on average higher in the control soils. Lastly, soils treated with compost had longer MRT of SOC.

Soil Moisture & Temperature

The soil moisture of the control soils at CH was significantly higher than the moisture found in the treatment soils. Moreover, the average soil moisture existing across sites varied significantly. At the CH site in particular, soil moisture was the primary driver of soil respiration. Soil moisture values were higher in the control plots than the treatment plots. The fluctuation in moisture over the growing season was also higher than the seasonal flux observed in the treatment plots. This within site variation was largely influenced by the experimental design and environmental conditions. The CH site runs parallel to an active creek approx. 200 meters to the south, Tomichi Creek, with the control plots existing closer to the river than the treatment plots. This influence of increased soil moisture in the control plots may present a skewed outcome when analyzing the impact of compost on soil respiration. It was observed in the early fall at CH that several control plots were inundated with water while the treatment plots remained dry, possibly due to the rising tide of the nearby river during this time of year. This site also did not receive any form of managed irrigation schedule or grazing regimes in the 2020 summer months, unlike the PP site that received both management implementations.

Over the 2020 growing season, the PP site received irrigation in the months of June and July, and was also subject to grazing and general disturbance by a rotation of horses, cows, sheep and alpacas. At the WL site, irrigation was implemented from approximately May 20, 2020 – July 10, 2020, yet received no disturbance from grazing. Similarly, CH received no grazing over the 2020 growing season, the sites management history however may shed some light as to the amount of organic matter present as it was left fallow for 20+ years and received occasional grazing after becoming a conservation easement. Moreover, Cooper et al. (2021) found the largest increase in %SOM following compost amendment at the CH site. Yet only the WL site had significantly different soil moisture values between treatments, with the treatment soils

having higher values than the control soils (Cooper et al., 2021). However, one year post amendment, this observation of significantly increased soil moisture in treatment soils was not seen.

Although not significant, PP treatment soils also had consistently higher values of soil moisture than the control soils. The CH treatment soils began with higher soil moisture, yet the control soils became higher in the months of September and October. These 2019 CH findings are drastically different from the findings observed in 2020. It is possible that the creek's rising tide coincided with the treatment soil's decrease in water holding capacity, with the control soils having higher soil moisture values beginning in September 2019, whereby this dominance is then observed throughout the entire 2020 growing season.

Soil moisture and soil respiration were positively correlated, a relationship that has been widely observed (Rey et al., 2002; Borken et al., 2006). Hursh et al., (2016) found that biomes with the highest mean soil carbon content had the lowest rates of soil respiration, indicating that respiration is less limited by carbon content and more controlled via the interaction of soil moisture and temperature. These interactive effects were also observed across the three research sites in this study. Whereby, a positive correlation was found between soil respiration and soil moisture. Yet contrary to prior expectations, a negative relationship was found for soil respiration and soil temperature. This relationship is best explained by the interaction of soil moisture and soil temperature at these sites. Soil moisture and soil temperature also had a negative relationship. In semi-arid ecosystems, soil respiration is more highly influenced by precipitation events than soil temperature (Moyes & Bowling, 2013). When soil temperature was high, respiration and moisture were low, therefore soil moisture exerted a stronger influence on respiration as it was limited at higher temperatures.

Microbial Community Composition

Treatment showed no significant effect on the biomass of any microbial functional group. It is possible that changes in the soil microbial community may need more than one year post compost amendment to detect. A lack of immediate shift in microbial community composition following compost amendments has been previously observed in a grassland ecosystem in Australia 3 months post amendment (Ng et al., 2015). On the other hand, following the yearly application of straw residues to the fluvo-aquic soils in the Northern Plains of China for 27 consecutive years, Zhang et al. (2019) found increases in the abundances of GP bacteria, fungi and anaerobes. The large breadth between these examples highlights the need for experimentation that frequently and regularly assesses microbial community composition over an extended time scale.

One of the longest microbial community composition studies found relating to this research was Sullivan et al. (2006) who tested microbial community composition on a semi-arid rangeland site in northern Colorado at two different time points. Ten years following a single biosolid application Sullivan et al. (2006) found no difference in fungi or bacteria abundance. They did, however, find enhanced microbial activity in mineralization of C and N and increased plant productivity when soil moisture was sufficient, indicating that perhaps the shift in community composition may not play as large of a role in increasing carbon sequestration compared to the role of increased microbial activity, soil moisture and plant productivity (Sullivan et al., 2006). This is supported by the findings from the CH site, where high microbial respiration was correlated with high soil moisture, yet the abundance and composition of the microbial community was unaffected by either soil moisture or nutrient availability.

Although the abundance of fungi or bacteria did not differ across sites or between treatments, their ratios did. Fungi to bacteria ratios were significantly different across sites, with generally higher values occurring at the CH and WL sites, and lower values at the PP site. The varying soil moisture contents found at each site may explain the variations observed in fungal and bacterial abundances. Because fungal communities have a high plasticity for soil moisture fluctuation, fungi are better adapted to soil drought than bacteria (Yuste et al., 2011; Kaisermann et al., 2015). Therefore, in soils experiencing prolonged drought conditions, such as WL, we would expect higher F:B ratios than soils experiencing a lower degree of drought stress, such as PP (Swift et al., 1979).

Drought severity can be analyzed using soil moisture percentiles, whereby 21 – 30% is classified as Abnormally Dry, 11 – 20% classified as Moderate Drought and 6 – 10% classified as Severe Drought (droughtmonitor.unl.edu). Across treatments, WL soil moisture consistently maintained moderate drought conditions, while PP soil moisture transitioned from severe drought conditions in mid-August to moderate drought conditions in late September. CH treatment soils maintained moderate drought conditions throughout the growing season, while the control soils only entered these conditions in late August. Thus, such variations in each site's moisture regimes could be a possible explanation for the variation in F:B ratios observed. The western United States is projected to experience more frequent and severe drought conditions and decrease in snowpack, therefore, it is possible that future F:B ratios may be more heavily influenced by environmental conditions (Zhang et al., 2021; Siirila-Woodburn et al., 2021).

Presently, the influence of environmental conditions on F:B ratios is well studied, however more research needs to be done examining the influence of compost on F:B ratios in rangeland soils. Bastida et al. (2008) found that semiarid soils amended with a sewage-sludge based compost

showed no shift in F:B ratios two years post application. The lack of change in F:B ratios following compost amendment in this study could be due to the timing at which soil samples were collected. Bacteria are more dominant in the spring and fall when vegetation is less active and/or present (Bardgett et al., 1999). In this study, soil samples were collected in the fall when fungal community abundance may not have been fully represented. Moreover, fungal communities are also supported by minimal soil disturbance and organic inputs (Teague et al., 2011; Gravuer et al., 2019). PP had the highest level of disturbance with a variety of grazing species present on a rotating basis, which could explain the generally lower values of F:B found.

Although the Bastida et al. (2008) study did not find differences in F:B following compost addition, they did find an increase in the GP:GN ratio of the amended soils 2 years post application compared to the unamended soil. Additionally, Zhang et al., (2019) found higher GP:GN ratios in soils with high nutrient levels compared to soils with lower nutrient levels. No significant difference was found in the GP:GN ratio of our study. However, there was a general trend for a higher abundance of GN dominated soils. The lowest GP:GN value observed was 0.4181(ng/g) indicating a GN dominated soil, while 2.234 (ng/g) was the highest, indicating a slightly GP dominated soil. There were no observations of a highly GP dominated soil in any of the sites. Due to their ability to produce spores, GP dominance is indicative of soils experiencing stress factors such as drought or extreme temperatures (Smit et al., 2001). The lack of strong GP dominance could indicate that, across sites, extreme stress factors were either missing or too weak to impact the bacterial community composition. GN dominance however is indicative of soils with heavy metal contamination, heavy pesticide application or anaerobic conditions (Storey et al., 2018). Heavy metals or pesticide applications are unlikely at these sites however, the CH control soils had a high degree of soil moisture, occasionally over 50% VWC which is

considered an inundated soil that could produce anaerobic conditions. Yet the opposite is observed whereby the GP:GN ratios of the CH control soils were generally considered a balanced bacterial community, indicating that perhaps anaerobic soils conditions were not dominant at this site. Overall, the bacterial community showed no change following compost addition.

Similarly, there was no change in the total microbial biomass found at each site. In a meta-analysis examining compost amendments to rangeland soils, Gravuer et al. (2019) found that compost amendments led to an overall increase in microbial biomass. The lowest and highest values for total PLFA biomass were both obtained in PP soils. This variation in concentrations obtained could indicate a high degree of spatial heterogeneity in community composition within this site. Although not significant, generally higher concentrations of protozoa were also found in the treatment soils at the PP site. Because protozoa feed on bacteria releasing nutrients to the soil, a higher ratio of protozoa to bacteria is indicative of soils that have a base level of nutrients high enough to support higher trophic levels (Esteban et al., 2006). Therefore, it is possible that the compost amendments at this site could have influenced the trend for more abundant protozoa, as compost amendments increase soil nutrient levels.

Similarly, the treatment soils at CH and PP sites exhibited generally higher abundances of arbuscular mycorrhizae, total GP bacteria, total GN bacteria and overall total PLFAs. Arbuscular mycorrhizae are important for litter decomposition and soil aggregate formation, two processes that are essential for carbon sequestration to take place (Al-Maliki et al., 2021). Zhang et al., (2016) found that inoculation with arbuscular mycorrhizae led to a decrease in soil CO_2 release and soil C depletion in semi-arid soils. If this trend continues, it could present an opportunity to further increase the amount of C sequestration following compost amendments.

Soil Respiration

Over the growing season, soil respiration rates varied across the three sites and also at each time point that was sampled. Although not significant, the control soils at CH exhibited slightly higher rates of soil respiration than the treatment soils. This observation is contrary to prior expectations that increased soil respiration would be found in the treatment soils, as increased nutrient availability should stimulate microbial activity (Fontaine et al., 2011; Luo et al., 2016). Additionally, after extrapolating daily efflux values and calculating the cumulative release of C-CO_2 over the entire growing season at all sites, the control soils exhibited significantly higher rates of efflux than did the treatment soils overall.

One possible explanation for the higher respiration rates found in the control soils could be due to the high correlation between soil respiration and soil moisture found across all sites. It is largely known that in general, soil moisture and soil respiration have a linearly positive relationship (Cook & Orchard, 2008). At the CH site, in particular, the control soils exhibited consistently higher values of VWC than the treatment soils throughout the growing season. Whereby, at the PP and WL sites, similar soil moisture and soil respiration values were observed between the control and treatment soils throughout the growing season. Even though a significant difference was not observed between treatments at the CH site, a general trend was observed supporting the theory that soil moisture was the driving factor influencing soil respiration in this study.

This influence of site variability is also exhibited in the timing at which each site reached peak efflux. The variation in peak efflux is important for understanding when the soil microbial community shifts from processing labile C to non-labile C. Whereby, the breakdown of labile C initially stimulates microbial activity in support of C sequestration, yet the processing of non-

labile C supports an overall loss of soil C to the atmosphere. This occurs at the PP and WL sites in the early summer, however this switch occurs at the CH site in late summer which could be attributed to the deeper organic layer existing at CH than at PP or WL. Moreover, the CH soils contained higher initial amounts of labile C, which could account for the extended labile C processing time observed.

Overall, soil respiration is the combined efflux of CO_2 from both heterotrophic respiration and plant root respiration. An increase in plant productivity will lead to an increase in both plant biomass and root biomass. Therefore, increased plant biomass resulting from compost amendments could also increase root respiration and thus the release of CO_2 to the atmosphere. Cooper et al. (2021) found significant increases in plant biomass at all three sites within just one month following compost amendment. Yet our findings showed the control soils exhibiting significantly higher rates of cumulative $C-CO_2$ efflux over the growing season compared to treated soils. Therefore, it is possible that the influence of increased plant productivity and root respiration from compost amendments was not influential enough to make a significant impact on total CO_2 efflux one year post application.

Ryals and Silver (2013) found that a single application of green waste compost increased CO_2 release over a three-year period, but also that this increased microbial respiration was offset by the increase in net plant productivity. In essence, the magnitude of CO_2 absorbed by the plants was larger than the amount released from the microbes; supporting the theory that compost can increase carbon sequestration in a relatively short amount of time. In general, however, Gravuer et al. (2019) found in a meta-analysis of compost amendments to rangeland soils that there is a general lack of studies addressing the long-term effects of compost amendments on climate

mitigation. This lack of knowledge highlights the importance of supporting the study of single

compost applications to rangeland soils, collecting data from multiple and ongoing time points.

Soil C & N Pools and Mean Residence Time

The MRT values ranged from a minimum of 0.64 years to a maximum of 3.21 years. These

values are relatively low when compared to global studies. Chen et al. (2020) estimated MRT of

SOC to range from 0.47 – 56.75 years across global biomes. However, in this study MRT values

were calculated based on respiration rates taken over a single growing season. It is possible that

MRT may be longer in the winter months. Therefore, by only assessing the growing season, the

reported MRT values could be an underestimate of the true annual MRT of SOC at these sites.

Moreover, it has been observed that German grassland ecosystems have an average MRT of

SOC of 29 years (Poeplau et al., 2021). The driving factor increasing MRT of SOC in such

grassland ecosystems, compared to other biomes, was the influence of increased root-derived

organic C inputs (Poeplau et al., 2021). In essence, the C pool exerted a stronger influence on

MRT of SOC than did the CO_2 efflux. Contrary to our findings, where the CO_2 flux exerted a

stronger influence on the MRT of SOC than did the C pool.

Soils treated with compost had longer MRT of SOC than soils not treated with compost. This

effect has been previously observed whereby compost additions to annual grassland soils

increased net SOC storage (Ryals & Silver, 2013). As the carbon pools did not significantly

differ between treatments, the difference in C-CO_2 efflux between treatments was responsible for

the differences observed in MRT of SOC. The control soils had higher rates of C-CO_2 efflux

over the growing season, resulting in shorter MRT of SOC. This finding is contrary to prior

expectations as nutrient inputs should have an effect of increased microbial activity and

respiration of CO_2 (Sullivan, 2006; Zhang et al., 2013).

Although the C and N pools did not significantly differ between treatments, the C:N ratios were significantly higher in the control soils. This is contrary to expectations as the C:N ratio of the compost was relatively high. This may indicate that the treatment soils experienced less N limitation, thereby utilizing more of the N pool and supporting the increased plant productivity that was found at all three sites four months post compost application (Cooper et al., 2021). In general, compost additions support increased plant productivity directly via the increase in soil N. Zhou et al. (2018) found that high plant diversity and plant productivity supported the accumulation of SOC via the influence of increased litter quality. It is possible that the combination of increased plant productivity and increased nutrient utilization influenced the longer MRT of SOC found in the composted soils.

Another possible explanation could be attributed to the strong influence that soil moisture had on soil respiration at these sites. Controls soils at the CH site had significantly higher levels of soil moisture than the treatment soils. The CH site also showed the largest difference in cumulative C-CO_2 efflux observed between treatments, with efflux being much higher in the control soils. This effect has been previously observed whereby abundant precipitation accelerated soil C decomposition producing faster C turnover times (Chen et al., 2013). However, when compared to mean annual temperature and mean annual precipitation, soil N storage has been shown to exert a stronger influence on MRT of SOC across ecosystems (Chen et al., 2013).

It has also been observed that soil C storage is supported by soil N additions (Lu et al., 2011; Chen et al., 2013; Huang et al., 2020). Increased N concentrations can stimulate cellulolysis, accelerating the degradation of labile C (Sinsabaugh et al., 2005; Grandy et al., 2008). However, increased N concentrations can also inhibit lignin-degrading enzymes, decelerating the degradation of recalcitrant C (Sinsabaugh et al., 2005; Janssens et al., 2010). No relationship

between C and N pools was observed in this study, however, C pool size and $C-CO_2$ efflux were positively correlated in the treatment soils only. While cumulative $C-CO_2$ efflux was significantly higher in the control soils, it appears that C inputs still stimulated $C-CO_2$ efflux in the treatment soils, most likely resulting from an increase in microbial activity.

Moreover, in the treatment soils only, C:N ratios were positively correlated with MRT and negatively correlated with $C-CO_2$ efflux. Conen et al. (2008) observed the opposite effect whereby as C:N ratios decreased, SOC stability increased. Decreasing soil C:N ratios can be used as an indication that microbial activity is taking place (Kramer et al., 2003). Initially, it was expected that increased microbial activity in the treatment soils would lead to a higher $C-CO_2$ efflux than the controls soils. As a positive relationship between C pool and $C-CO_2$ efflux was observed in the treatment soils only, it can be inferred that microbial activity was indeed stimulated. Contrary to expectations, however, the control soils exhibited higher cumulative $C-CO_2$ efflux than the treatment soils. Indicating that through an increase in microbial activity, one-year post compost amendment, SOC was sequestered in the treatment soils for a longer period of time than in the soils not amended with compost. The MRT values obtained in this study are relatively low, Lal et al. (2015) estimates that MRT values need to be on the order of decades to millennia to make a significant impact on the reduction of global atmospheric CO_2. However, this study gives a representation of the influence that compost can have on soil C sequestration one year post application, providing a baseline used in determining the impacts to be expected with the scaling up of such land management practices on rangelands for the sequestration of global atmospheric carbon.

Management Implications

Compost applications to rangeland soils can help mitigate climate change by increasing soil carbon sequestration. Composting can also increase plant productivity providing direct benefit to ranchers and their cattle. Ranchers may also benefit from the use of this data in connection with carbon market programs. For example, the Savory Institute implements a program whereby ranchers are rewarded if they can provide verifiable results that their management techniques are improving ecological health (https://savory.global/land-to-market/eov/). Additionally, under California's cap and trade programs, ranchers can receive carbon credits if they can demonstrate that their land is sequestering carbon (https://www.c2es.org/content/california-cap-and-trade/). For the best results, ample irrigation is necessary. Special care must also be taken when scaling up this strategy that CO_2 emissions released during transportation of the compost does not offset the amount of C being sequestered in the soil. This study is unique, however, as the compost was locally sourced and provides a relatively inexpensive and sustainable source of compost for ranchers in this area.

Specifically, when Gunny Gold compost was applied to rangeland soils at a 5 cm thickness level, carbon storage was observed after one year. This compost is a low cost and locally sourced option for land managers of the Gunnison Valley, and is also rich in nitrogen, phosphorus and potassium, essential nutrients that support plant growth. Thereby feed for cattle is an additional co-benefit received by these amendments. Using the municipal waste compost amendment across the valley would provide the co-benefits of reducing greenhouse gas emissions associated with such waste, recycling the waste in a responsible manner while also improving soil health and increasing feed for cattle. Applied broadly, this technique will have higher effectiveness. With

increased soil health, carbon storage and plant productivity, the benefits of this technique outweigh the costs in perennial grasslands.

Conclusions

We found that a single application of compost to rangeland soils in the Gunnison Valley of Colorado increased the MRT of SOC. No shifts in microbial communities were observed, it may be that these shifts require longer than one year post application to detect. Soil moisture exerted a stronger influence on soil respiration than did soil temperature. Compost treatment has no effect on daily average respiration rates, however the cumulative release of C-CO_2 over the growing season was higher in the non-composted soils. As the C pools were not significantly different between treatments, the significance lies in the difference in efflux observed. Overall, the three main takeaways from this research lie in the influence that compost had on carbon storage within the perennial grasslands of the Gunnison Valley. Firstly, the MRT of SOC can be increased through the use of compost amendments. Secondly, compost additions stimulated soil C processing, yet decreased soil respiration. Thirdly, following compost amendment, after one year, the overall support of soil health and plant productivity supported the sequestration of atmospheric carbon. Therefore, this management strategy may be used to increase soil C sequestration in rangeland soils thereby supporting the mitigation of climate change.

Future Research

This study highlights the need for more in-depth and longer lasting research studying soil carbon storage. This research could be further strengthened through several series of more in-depth

analysis to understand the deeper mechanisms at play as to the relationships between microbial activity, soil respiration and nutrient cycling. The higher flux observed in the control soils was an unexpected outcome of this study that could be better understood with an incubation study testing efflux over time at varying levels of nutrient inputs. An incubation study could also be conducted to assess the contribution that root respiration adds to the overall soil respiration. Assessing the amount of carbon and nitrogen within the biomass of the soil microbes would provide insight as to the level of activity that occurred as well. Furthermore, analysis of the plant tissues for their composition of nutrients would provide insight into the role of plant productivity and nutrient cycling in the process of increasing carbon storage.

Acknowledgements

Literature Cited

Al-Maliki, S., Al-Amery, A., Sallal, M., & Al-Taey, D. K. A. (2021). Effects of Arbuscular
    Mycorrhiza and Organic Wastes on Soil Carbon Mineralisation, Actinomycete sand
    Nutrient Content in Maize Plants (Zea Mays L.). 25, 19.

Bailey, V. L., Smith, J. L., & Bolton, H. (2002). Fungal-to-bacterial ratios in soils investigated
    for enhanced C sequestration. *Soil Biology and Biochemistry*, *34*(7), 997–1007. doi:
    10.1016/S0038-0717(02)00033-0

Bardgett, R. D., Lovell, R. D., Hobbs, P. J., & Jarvis, S. C. (1999). Seasonal changes in soil
    microbial communities along a fertility gradient of temperate grasslands. *Soil Biology
    and Biochemistry*, *31*(7), 1021–1030. https://doi.org/10.1016/S0038-0717(99)00016-4

Bastida, F., Kandeler, E., Moreno, J. L., Ros, M., García, C., & Hernández, T. (2008).
    Application of fresh and composted organic wastes modifies structure, size and activity
    of soil microbial community under semiarid climate. *Applied Soil Ecology*, *40*(2), 318–
    329. doi: 10.1016/j.apsoil.2008.05.007

Bastida, Felipe, Hernández, T., Albaladejo, J., & García, C. (2013). Phylogenetic and functional

changes in the microbial community of long-term restored soils under semiarid climate.

Soil Biology and Biochemistry, *65*, 12–21. doi: 10.1016/j.soilbio.2013.04.022

Beck, T., R. G. Joergensen, E. Kandeler, F. Makeschin, E. Nuss, H. R. Oberholzer, and S. Scheu.

1997. An inter-laboratory comparison of ten different ways of measuring soil microbial

biomass C. Soil Biol. Biochem. 29 (7):1023-1032.

Bhatti, A. A., Haq, S., & Bhat, R. A. (2017). Actinomycetes benefaction role in soil and plant

health. *Microbial Pathogenesis*, *111*, 458–467.

https://doi.org/10.1016/j.micpath.2017.09.036

Billings, H., poWell, M., & Doucette, K. (2000). Robin White Pilot Analysis of Global

Ecosystems Grassland Ecosystems Grassland Ecosystems Pilot Analysis of Global

Ecosystems (page) Coastal Ecosystems Forest Ecosystems Freshwater Systems Grassland

Ecosystems Project Management Forest Ecosystems Grassland Storage of C C Stores in

Grasslan. Retrieved April 13, 2020, from https://www.semanticscholar.org/paper/Robin-

White-Pilot-Analysis-of-Global-Ecosystems-of-Billings-

poWell/e2a06b1c954a7691a526abb836538a0e0d0c411f

Borken, W., Savage, K., Davidson, E. A., & Trumbore, S. E. (2006). Effects of experimental

    drought on soil respiration and radiocarbon efflux from a temperate forest soil: drought

    effects on soil respiration. *Global Change Biology*, *12*(2), 177–193.

    https://doi.org/10.1111/j.1365-2486.2005.001058.x

Chapin, F. S., Matson, P. A., & Vitousek, P. M. (2011). *Principles of Terrestrial Ecosystem*

    Ecology. Springer New York. https://doi.org/10.1007/978-1-4419-9504-9

Chen, S., Huang, Y., Zou, J., & Shi, Y. (2013). Mean residence time of global topsoil organic C

    depends on temperature, precipitation and soil nitrogen. *Global and Planetary Change*,

    100, 99–108. doi: 10.1016/j.gloplacha.2012.10.006

Chen, S., Zou, J., Hu, Z., & Lu, Y. (2020). Temporal and spatial variations in the mean residence

    time of soil organic carbon and their relationship with climatic, soil and vegetation

    drivers. *Global and Planetary Change*, *195*, 103359.

    https://doi.org/10.1016/j.gloplacha.2020.103359

Conen, F., Zimmermann, M., Leifeld, J., Seth, B., & Alewell, C. (2008). Relative stability of soil

    carbon revealed by shifts in $\delta15N$ and C:N ratio. 6.

Cooper, A., & DeMarco, J. (2021). Grassland Resilience through Biosolids Compost
Amendments: Supporting soil health for improved community adaptation and climate
mitigation. *Manuscript in Progress*.

Fanin, N., Kardol, P., Farrell, M., Nilsson, M.-C., Gundale, M. J., & Wardle, D. A. (2019). The
ratio of Gram-positive to Gram-negative bacterial PLFA markers as an indicator of
carbon availability in organic soils. *Soil Biology and Biochemistry*, *128*, 111–114.
https://doi.org/10.1016/j.soilbio.2018.10.010

Fierer, N., Schimel, J. P., & Holden, P. A. (2003). Variations in microbial community
composition through two soil depth profiles. *Soil Biology and Biochemistry*, *35*(1), 167–
176. https://doi.org/10.1016/S0038-0717(02)00251-1

Fontaine, S., Henault, C., Aamor, A., Bdioui, N., Bloor, J. M. G., Maire, V., Mary, B., Revaillot,
S., & Maron, P. A. (2011). Fungi mediate long term sequestration of carbon and nitrogen
in soil through their priming effect. *Soil Biology and Biochemistry*, *43*(1), 86–96.
https://doi.org/10.1016/j.soilbio.2010.09.017

Global Carbon Budget 2020, by Pierre Friedlingstein, Michael O'Sullivan, Matthew W. Jones,
Robbie M. Andrew, Judith Hauck, Are Olsen, Glen P. Peters, Wouter Peters, Julia
Pongratz, Stephen Sitch, Corinne Le Quéré, Josep G. Canadell, Philippe Ciais, Robert B.

Jackson, Simone Alin, Luiz E. O. C. Aragão, Almut Arneth, Vivek Arora, Nicholas R. Bates, Meike Becker, Alice Benoit-Cattin, Henry C. Bittig, Laurent Bopp, Selma Bultan , Naveen Chandra, Frédéric Chevallier, Louise P. Chini, Wiley Evans, Liesbeth Florentie, Piers M. Forster, Thomas Gasser, Marion Gehlen, Dennis Gilfillan, Thanos Gkritzalis, Luke Gregor, Nicolas Gruber, Ian Harris, Kerstin Hartung, Vanessa Haverd, Richard A. Houghton, Tatiana Ilyina, Atul K. Jain, Emilie Joetzjer, Koji Kadono, Etsushi Kato, Vassilis Kitidis, Jan Ivar Korsbakken, Peter Landschützer, Nathalie Lefèvre, Andrew Lenton, Sebastian Lienert, Zhu Liu, Danica Lombardozzi, Gregg Marland, Nicolas Metzl, David R. Munro, Julia E. M. S. Nabel, Shin-Ichiro Nakaoka, Yosuke Niwa, Kevin O'Brien, Tsuneo Ono, Paul I. Palmer, Denis Pierrot, Benjamin Poulter, Laure Resplandy, Eddy Robertson, Christian Rödenbeck, Jörg Schwinger, Roland Séférian, Ingunn Skjelvan, Adam J. P. Smith, Adrienne J. Sutton, Toste Tanhua, Pieter P. Tans, Hanqin Tian, Bronte Tilbrook, Guido van der Werf, Nicolas Vuichard, Anthony P. Walker, Rik Wanninkhof, Andrew J. Watson, David Willis, Andrew J. Wiltshire, Wenping Yuan, Xu Yue, and Sönke Zaehle (2020), Earth System Science Data, 12, 3269–3340, 2020, DOI: 10.5194/essd-12-3269-2020.

Grandy, A. S., Sinsabaugh, R. L., Neff, J. C., Stursova, M., & Zak, D. R. (2008). Nitrogen deposition effects on soil organic matter chemistry are linked to variation in enzymes, ecosystems and size fractions. *Biogeochemistry*, *91*(1), 37–49. https://doi.org/10.1007/s10533-008-9257-9

Gravuer K, Gennet S, Throop HL. Organic amendment additions to rangelands: A meta-analysis of multiple ecosystem outcomes. Glob Change Biol. 2019;25:1152–1170. https://doi.org/10.1111/gcb.14535

Gude, A., Kandeler, E., & Gleixner, G. (2012). Input related microbial C dynamic of soil organic matter in particle size fractions. *Soil Biology and Biochemistry*, *47*, 209–219. doi:10.1016/j.soilbio.2012.01.003

Gunnison Gold Compost. Gunnison County Public Works. https://www.gunnisonco.gov/departments/public_ works/wastewater_treatment_plant/gunnison_gold_compost.php

Hamdali, H., Hafidi, M., Virolle, M. J., & Ouhdouch, Y. (2008). Rock phosphate-solubilizing Actinomycetes: Screening for plant growth-promoting activities. *World Journal of Microbiology and Biotechnology*, *24*(11), 2565–2575. https://doi.org/10.1007/s11274-008-9817-0

Hill, P. W., Farrar, J. F., & Jones, D. L. (2008). Decoupling of microbial glucose uptake and mineralization in soil. *Soil Biology and Biochemistry*, *40*(3), 616–624. doi: 10.1016/j.soilbio.2007.09.008

Huang, X., Terrer, C., Dijkstra, F.A. *et al*.(2020). New soil carbon sequestration with nitrogen

    enrichment: a meta-analysis. *Plant Soil* 454, 299–310.

    https://doi.org/10.1007/s11104-020-04617-x

Hursh, A., Ballantyne, A., Cooper, L., & Maneta, M. (2016). The sensitivity of soil respiration to

    soil temperature, moisture, and carbon supply at the global scale. *Global Change*

    Biology, 14.

Jagadamma, S., Steinweg, J. M., Mayes, M. A., Wang, G., & Post, W. M. (2014).

    Decomposition of added and native organic C from physically separated fractions of

    diverse soils. *Biology and Fertility of Soils*, *50*(4), 613–621. doi: 10.1007/s00374-013-

    0879-2

Janssens IA, Dieleman W, Luyssaert S, Subke JA, Reichstein M, Ceulemans R, Ciais P, Dolman

    AJ, Grace J, Matteucci G, Papale D, Piao SL, Schulze ED, Tang J, Law BE (2010)

    Reduction of forest soil respiration in response to nitrogen deposition. Nat Geosci 3:315–

    322.

Jastrow, J. D., Amonette, J. E., & Bailey, V. L. (2007). Mechanisms controlling soil C turnover

    and their potential application for enhancing C sequestration. *Climatic Change*, *80*(1–2),

    5–23. doi: 10.1007/s10584-006-9178-3

Kaisermann, A. (2015). Fungal communities are more sensitive indicators to non-extreme soil

    moisture variations than bacterial communities. *Applied Soil Ecology*, 7.

Kato, K., & Miura, N. (2008). Effect of matured compost as a bulking and inoculating agent on

    the microbial community and maturity of cattle manure compost. *Bioresource*

    Technology, 9.

Knorr, W., Prentice, I. C., House, J. I., & Holland, E. A. (2005). Long-term sensitivity of soil

    carbon turnover to warming. *Nature*, *433*(7023), 298–301.

    https://doi.org/10.1038/nature03226

Kong, A. Y. Y., Scow, K. M., Córdova-Kreylos, A. L., Holmes, W. E., & Six, J. (2011).

    Microbial community composition and C cycling within soil microenvironments of

    conventional, low-input, and organic cropping systems. *Soil Biology and Biochemistry*,

    43(1), 20–30. doi: 10.1016/j.soilbio.2010.09.005

Lal, R. (2007). Anthropogenic Influences on World Soils and Implications to Global Food

    Security. In *Advances in Agronomy* (Vol. 93, pp. 69–93). Elsevier.

    https://doi.org/10.1016/S0065-2113(06)93002-8

Lal, R., Negassa, W., & Lorenz, K. (2015). Carbon sequestration in soil. *Current Opinion in*

Environmental Sustainability, *15*, 79–86. https://doi.org/10.1016/j.cosust.2015.09.002

Lohila, A., Aurela, M., Regina, K., & Laurila, T. (2003). Soil and total ecosystem respiration in agricultural fields: Effect of soil and crop type. *Plant and Soil*, *251*, 303–317.

Lu M, Zhou X, Luo Y, Yang Y, Fang C, Chen J, Li B (2011) Minor stimulation of soil carbon storage by nitrogen addition: a meta-analysis. Agric Ecosyst Environ 140(1–2):234– 244

Luo, Z., Wang, E., & Sun, O. J. (2016). A meta-analysis of the temporal dynamics of priming soil carbon decomposition by fresh carbon inputs across ecosystems. *Soil Biology and Biochemistry*, *101*, 96–103. https://doi.org/10.1016/j.soilbio.2016.07.011

Maps | U.S. Drought Monitor, droughtmonitor.unl.edu/Maps.aspx.

McCarthy, A. J., & Williams, S. T. (1992). Actinomycetes as agents of biodegradation in the environment—A review. *Gene*, *115*(1–2), 189–192. https://doi.org/10.1016/0378-1119(92)90558-7

Moore, J. C., McCann, K., & de Ruiter, P. C. (2005). Modeling trophic pathways, nutrient cycling, and dynamic stability in soils. *Pedobiologia*, *49*(6), 499–510. doi: 10.1016/j.pedobi.2005.05.008

Moyes, A. B., & Bowling, D. R. (2013). Interannual variation in seasonal drivers of soil

    respiration in a semi-arid Rocky Mountain meadow. 15.

Morgan, J. A., Follett, R. F., Allen, L. H., Del Grosso, S., Derner, J. D., Dijkstra, F., …

    Schoeneberger, M. M. (2010). C sequestration in agricultural lands of the United States.

    Journal of Soil and Water Conservation, *65*(1), 6A-13A. doi: 10.2489/jswc.65.1.6A

Ng, E.-L., Patti, A. F., Rose, M. T., Schefe, C. R., Smernik, R. J., & Cavagnaro, T. R. (2015). Do

    organic inputs alter resistance and resilience of soil microbial community to drying? *Soil*

    Biology and Biochemistry, *81*, 58–66. https://doi.org/10.1016/j.soilbio.2014.10.028

"Plantmaps - Hardiness ZONE Maps and Much More." *Zdm*, www.plantmaps.com/.

Poeplau, C., Don, A., & Schneider, F. (2021). Roots are key to increasing the mean residence

time of organic carbon entering temperate agricultural soils. *Global Change Biology*, *27*(19),

4921–4934. https://doi.org/10.1111/gcb.15787

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for

    Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rey, A., Pegoraro, E., Tedeschi, V., De Parri, I., Jarvis, P. G., & Valentini, R. (2002). Annual

    variation in soil respiration and its components in a coppice oak forest in Central Italy:

    ANNUAL VARIATION IN SOIL RESPIRATION. *Global Change Biology*, *8*(9), 851–

    866. https://doi.org/10.1046/j.1365-2486.2002.00521.x

Ru, J., Zhou, Y., Hui, D., Zheng, M., & Wan, S. (2018). Shifts of growing-season precipitation

    peaks decrease soil respiration in a semiarid grassland. *Global Change Biology*, *24*(3),

    1001–1011. https://doi.org/10.1111/gcb.13941

Ryals, R., & Silver, W. L. (2013). Effects of organic matter amendments on net primary

    productivity and greenhouse gas emissions in annual grasslands. *Ecological Applications*,

    23(1), 46–59. doi: 10.1890/12-0620.1

Ryckeboer, J., Mergaert, J., Vaes, K., Klammer, S., Clercq, D. D., Coosemans, J., Insam, H., &

    Swings, J. (n.d.). A survey of bacteria and fungi occurring during composting and self-

    heating processes. 63.

Sinsabaugh, R. L., Gallo, M. E., Lauber, C., Waldrop, M. P., & Zak, D. R. (2005). Extracellular

    Enzyme Activities and Soil Organic Matter Dynamics for Northern Hardwood Forests

    receiving Simulated Nitrogen Deposition. *Biogeochemistry*, *75*(2), 201–215.

    https://doi.org/10.1007/s10533-004-7112-1

Savory Institute. "EOV: Measuring REGENERATIVE Outcomes for Food and FIBER ⋆ Savory

      Institute." *Savory Institute*, savory.global/land-to-market/eov/.

Siirila-Woodburn, E. R. (2021). A low-to-no snow future and its impacts on water resources in

the western United States. *Nature Reviews*, *2*, 20.

Silver, W. L., Vergara, S. E., & Mayer, A. (2018). California's Fourth Climate Change

      Assessment. *California Natural Resources Agency*, 62.

"Simply Precise: Meter Group." *METER*, 16 June 2020, www.metergroup.com/.

Simpson, R. T., Frey, S. D., Six, J., & Thiet, R. K. (2004). Preferential Accumulation of

      Microbial C in Aggregate Structures of No-Tillage Soils. *Soil Science Society of America*

      Journal, *68*(4), 1249– 1255. doi: 10.2136/sssaj2004.1249

Smit, E., Leeflang, P., Gommans, S., van den Broek, J., van Mil, S., & Wernars, K. (2001).

      Diversity and Seasonal Fluctuations of the Dominant Members of the Bacterial Soil

      Community in a Wheat Field as Determined by Cultivation and Molecular Methods.

      Applied and Environmental Microbiology, *67*(5), 2284–2291.

      https://doi.org/10.1128/AEM.67.5.2284-2291.2001

Soil Survey Staff, Natural Resources Conservation Service, United States Department of

Agriculture. Web Soil Survey. http://websoilsurvey.sc.egov.usda.gov/. Accessed

[12/18/2020].

"Stable Isotope Ecosystem Lab of Uc Merced." *Stable Isotope Ecosystem Lab of UC Merced*, 3

June 2021, isotopes.ucmerced.edu/.

Stefanis, C., Alexopoulos, A., Voidarou, C., Vavias, S., & Bezirtzoglou, E. (2013). Principal

methods for isolation and identification of soil microbial communities. *Folia

Microbiologica*, *58*(1), 61–68. doi: 10.1007/s12223-012-0179-5

Storey, S., Ashaari, M. M., Clipson, N., Doyle, E., & de Menezes, A. B. (2018). Opportunistic

Bacteria Dominate the Soil Microbiome Response to Phenanthrene in a Microcosm-

Based Study. *Frontiers in Microbiology*, *9*, 2815.

https://doi.org/10.3389/fmicb.2018.02815

Sullivan, T. S., Stromberger, M. E., & Paschke, M. W. (2006). Parallel shifts in plant and soil

microbial communities in response to biosolids in a semi-arid grassland. *Soil Biology and

Biochemistry*, *38*(3), 449–459. https://doi.org/10.1016/j.soilbio.2005.05.018

M. J. Swift, O. W. Heal, J. M. Anderson, Decomposition in Terrestrial Ecosystems (Blackwell, Oxford, 1979).

Teague, W. R., Dowhower, S. L., Baker, S. A., Haile, N., DeLaune, P. B., & Conover, D. M. (2011). Grazing management impacts on vegetation, soil biota and soil chemical, physical and hydrological properties in tall grass prairie. *Agriculture, Ecosystems & Environment*, *141*(3–4), 310–322. https://doi.org/10.1016/j.agee.2011.03.009

Torres, I. F., Bastida, F., Hernández, T., Albaladejo, J., & García, C. (2015). Enzyme activity, microbial biomass and community structure in a long-term restored soil under semi-arid conditions. *Soil Research*, *53*(5), 553. doi: 10.1071/SR14297

U.S. Climate Data, "Weather Averages Gunnison, Colorado." www.usclimatedata.com/climate/gunnison/colorado/united-states/usco0175. Accessed [12/18/2020].

Villa, Y. B., & Ryals, R. (2021). Soil carbon response to long-term biosolids application. *Journal of Environmental Quality*, *50*(5), 1084–1096. https://doi.org/10.1002/jeq2.20270

Wang, X., Min, F., Yu, D., Xin, Z., Li, L., Li, X., Sun, X., & Pan, J. (2021). Mean residence

    times of active and slow soil organic carbon pools in croplands across China. *CATENA*,

    202, 105271. https://doi.org/10.1016/j.catena.2021.105271

Ward Laboratories, Inc. Standard Operating Procedure 1111 – FAME and PLFA in Soil.

Yuste, J. C., Peñuelas, J., Estiarte, M., Garcia-Mas, J., Mattana, S., Ogaya, R., Pujol, M., &

    Sardans, J. (2011). Drought-resistant fungi control soil organic matter decomposition and

    its response to temperature: Fungal Diversity Control Over Soil C Balance. *Global*

    Change Biology, *17*(3), 1475–1486. https://doi.org/10.1111/j.1365-2486.2010.02300.x

Zhang, Q.-C., Shamsi, I. H., Xu, D.-T., Wang, G.-H., Lin, X.-Y., Jilani, G., … Chaudhry, A. N.

    (2012). Chemical fertilizer and organic manure inputs in soil exhibit a vice versa pattern

    of microbial community structure. *Applied Soil Ecology*, *57*, 1–8. doi:

    10.1016/j.apsoil.2012.02.012

Zhang, X. B., Xu, M. G., Sun, N., Wang, X. J., Wu, L., Wang, B. R., & Li, D. C. (2013). How do

    environmental factors and different fertilizer strategies affect soil CO2 emission and C

    sequestration in the upland soils of southern China? *Applied Soil Ecology*, *72*, 109–118.

    doi:10.1016/j.apsoil.2013.05.014

Zhang, L., Xu, M., Liu, Y., Zhang, F., Hodge, A., & Feng, G. (2016). Carbon and phosphorus

    exchange may enable cooperation between an arbuscular mycorrhizal fungus and a

    phosphate-solubilizing bacterium. *New Phytologist*, *210*(3), 1022–1032.

    https://doi.org/10.1111/nph.13838

Zhang, X., Xin, X., Yang, W., Zhu, A., & Ding, S. (2019). Short-term decomposition, turnover

    and retention of residue-derived C are influenced by the fertility level in a sandy loam

    soil. *Geoderma*, *349*, 68–78. doi: 10.1016/j.geoderma.2019.04.043

Zhang, F., Biederman, J. A., Dannenberg, M. P., Yan, D., Reed, S. C., & Smith, W. K. (2021).

    Five Decades of Observed Daily Precipitation Reveal Longer and More Variable Drought

    Events Across Much of the Western United States. *Geophysical Research Letters*, *48*(7).

    https://doi.org/10.1029/2020GL092293
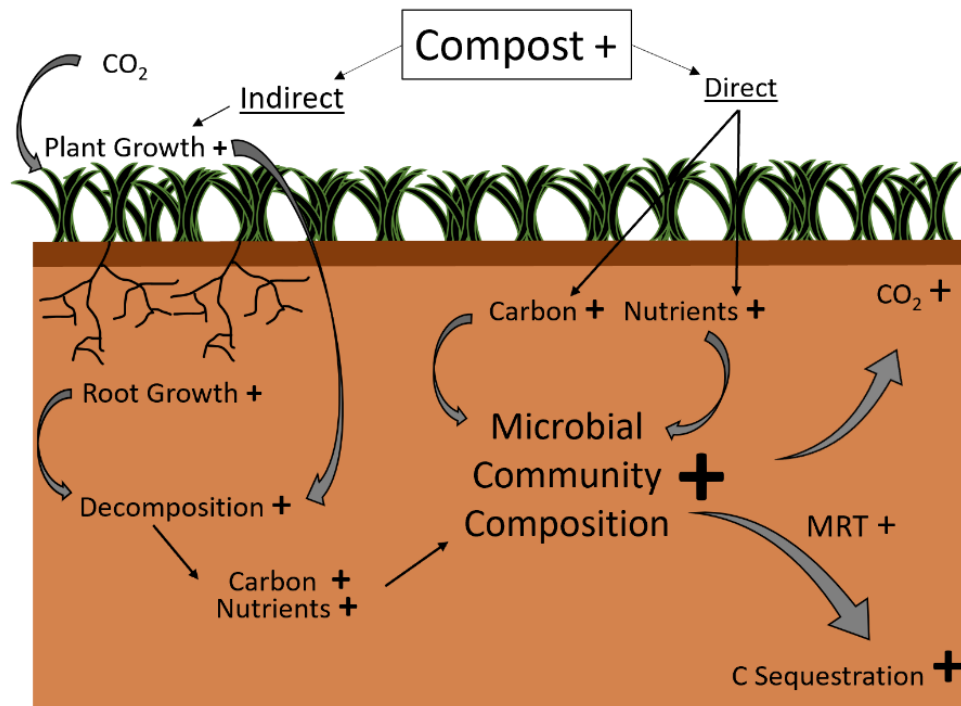
Figure 1. Conceptual diagram of the influence of compost additions on microbial community composition and C cycling. MRT = mean residence time.
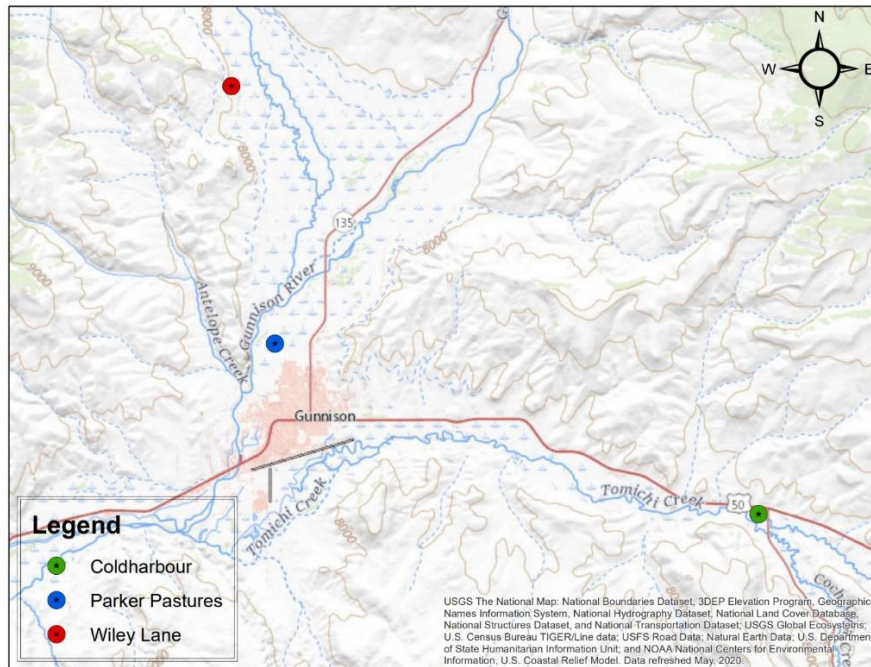
Figure 2. Map of the three rangeland research sites located in Gunnison county, including (1) Coldharbour (2) Parker Pastures and (3) Wiley Lane.
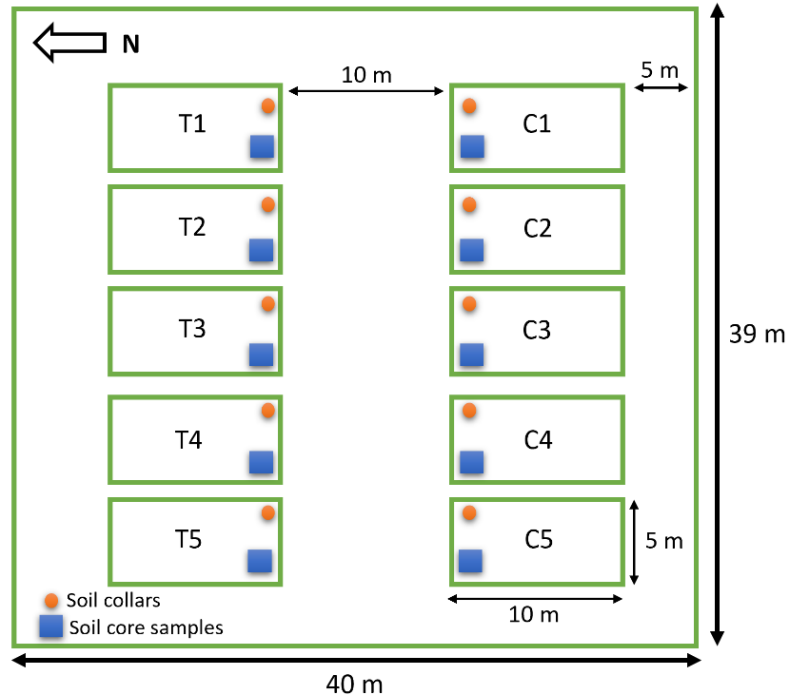
Figure 3. Outline and dimensions of each site, including 5 replicate plots per treatment. Orange circles indicate the locations of all soil collars. Blue squares indicate the position where soil cores were collected.
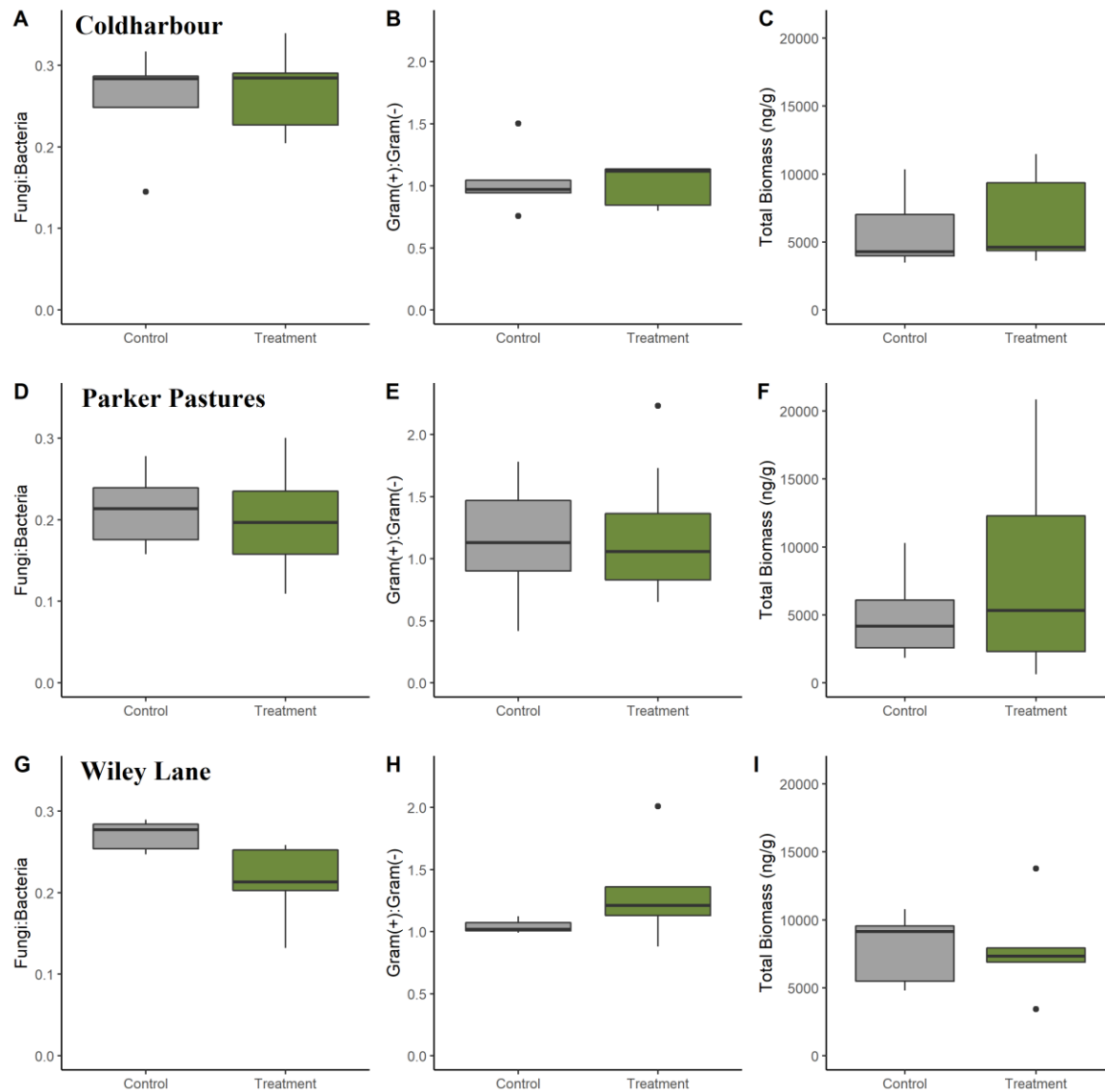
Figure 4. Mean ±SE ratios of Fungi:Bacteria, Gram(+):Gram(-) and total PLFA biomass (ng/g) found in the organic and mineral horizons of the control and treatment soils at the Coldharbour site (A-C); Parker Pastures site (D-F); and Wiley Lane site (G-I).

Table 1. Mean ± SE phospholipid fatty acid biomass concentrations of fungal and bacterial communities. Total n = 38 samples. PLFA biomass units: micrograms/gram. (A.M. = Arbuscular mycorrhizae; Sap. = Saprophytes; Act. = Actinomycetes). (C = Control; T = Treatment). (Gram + includes actinomycetes, Gram – includes rhizobia, Total Fungi is the sum of AB and Sap., Total bacteria is the sum of Gram + and Gram –). No significant differences were found within groups across sites or treatments.

| Site			n	Total Fungi	Total Bacteria	Gram +	Gram −	A.M.	Sap.	Act.	Rhizobia	Protozoa
CH	C	Org	5	0.7 (0.2)	2.6 (0.5)	1.3 (0.2)	1.3 (0.3)	0.3 (0.06)	0.4 (0.1)	0.4 (0.007)	0.004 (0.004)	0.01 (0.01)
		Min	0									
	T	Org	5	0.9 (0.3)	3.2 (0.8)	1.5 (0.3)	1.6 (0.4)	0.4 (0.1)	0.5 (0.1)	0.4 (0.09)	0.003 (0.002)	0.01 (0.007)
		Min	0									
PP	C	Org	4	0.6 (0.2)	2.7 (0.9)	1.2 (0.4)	1.5 (0.4)	0.2 (0.08)	0.4 (0.1)	0.4 (0.1)	0.0 (0.0)	0.02 (0.01)
		Min	4	0.3 (0.1)	1.6 (0.3)	0.9 (0.2)	0.6 (0.1)	0.1 (0.04)	0.2 (0.07)	0.3 (0.08)	0.004 (0.001)	0.007 (0.003)
	T	Org	5	2.0 (0.8)	7.7 (2.7)	3.3 (1.0)	4.3 (1.6)	0.8 (0.3)	1.2 (0.5)	1.1 (0.3)	0.02 (0.01)	0.1 (0.07)
		Min	5	0.3 (0.1)	1.8 (0.6)	0.9 (0.3)	0.8 (0.2)	0.1 (0.03)	0.2 (0.08)	0.3 (0.1)	0.005 (0.002)	0.006 (0.003)
WL	C	Org	0									
		Min	5	1.1 (0.1)	4.1 (0.4)	2.1 (0.2)	2.0 (0.2)	0.4 (0.04)	0.7 (0.08)	0.7 (0.07)	0.007 (0.004)	0.03 (0.01)
	T	Org	0									
		Min	5	0.8 (0.2)	3.5 (0.8)	1.8 (0.3)	1.6 (0.4)	0.3 (0.08)	0.5 (0.1)	0.6 (0.1)	0.006 (0.004)	0.03 (0.01)
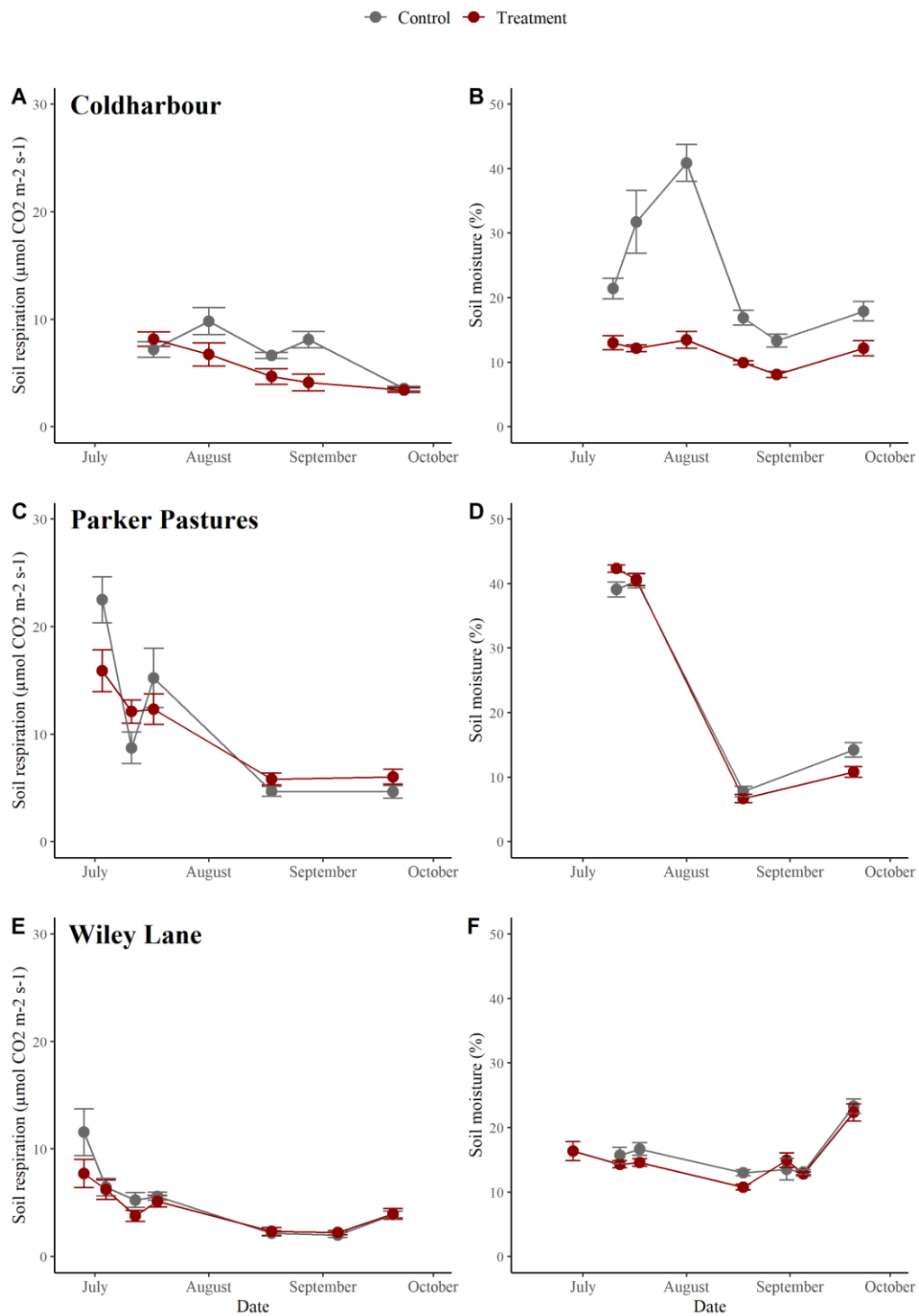
Figure 5. Mean ± SE soil respiration and volumetric water content for the sites: (A, B) Coldharbour; (C, D) Parker Pastures; (E, F) Wiley Lane.
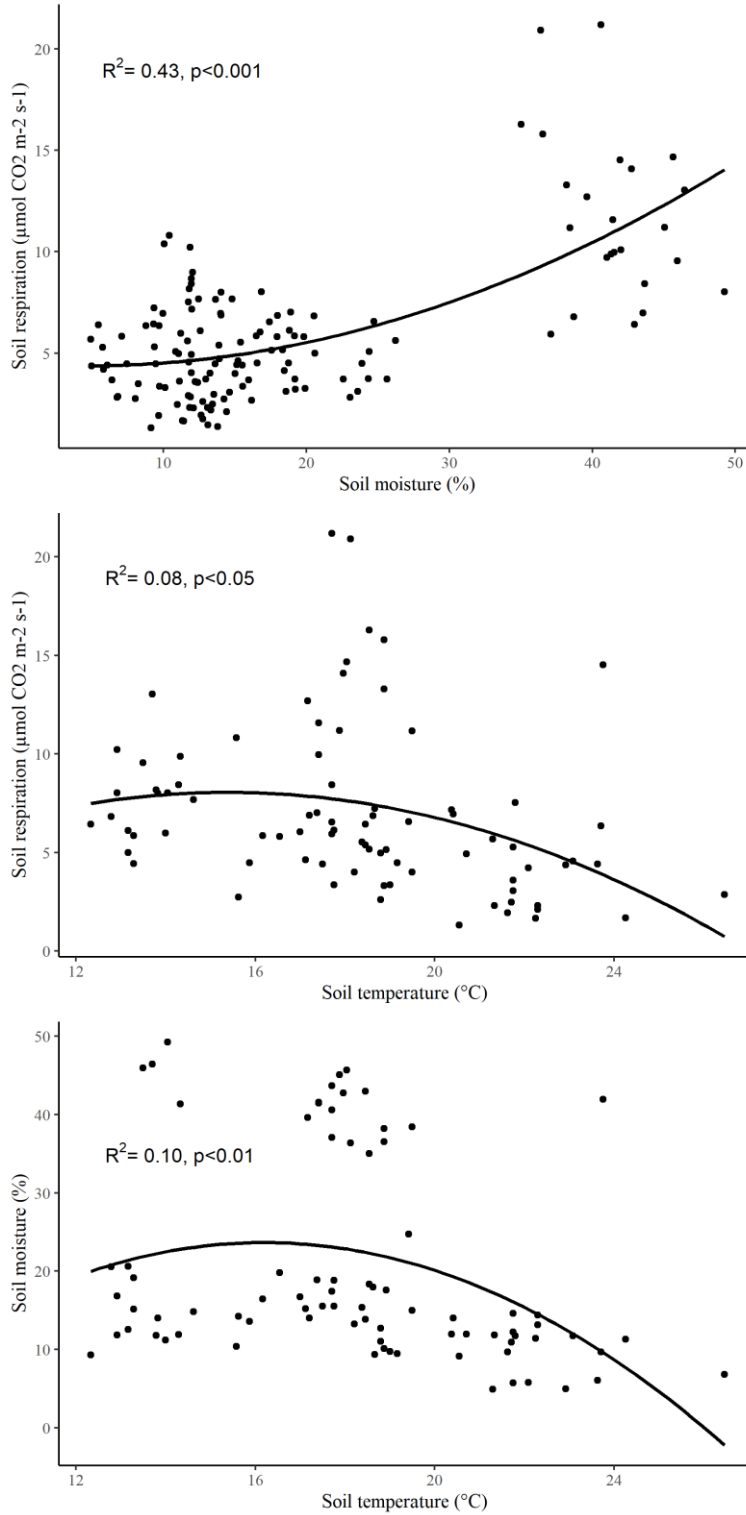
Figure 6. Relationships between soil respiration rates, soil volumetric water content (0 – 20 cm) and soil temperature (3 cm) in all treatments at all sites.

Table 2. Mean ± SE Bulk Density (g/cm^3), % Nitrogen, % Carbon, Nitrogen Pool (kg N/m2), Carbon Pool (kg C/m2) and Carbon:Nitrogen ratio within each treatment at each site. (C = Control, T = Treatment, CH = Coldharbour, PP = Parker Pastures, WL = Wiley Lane). Total n = 43 samples.

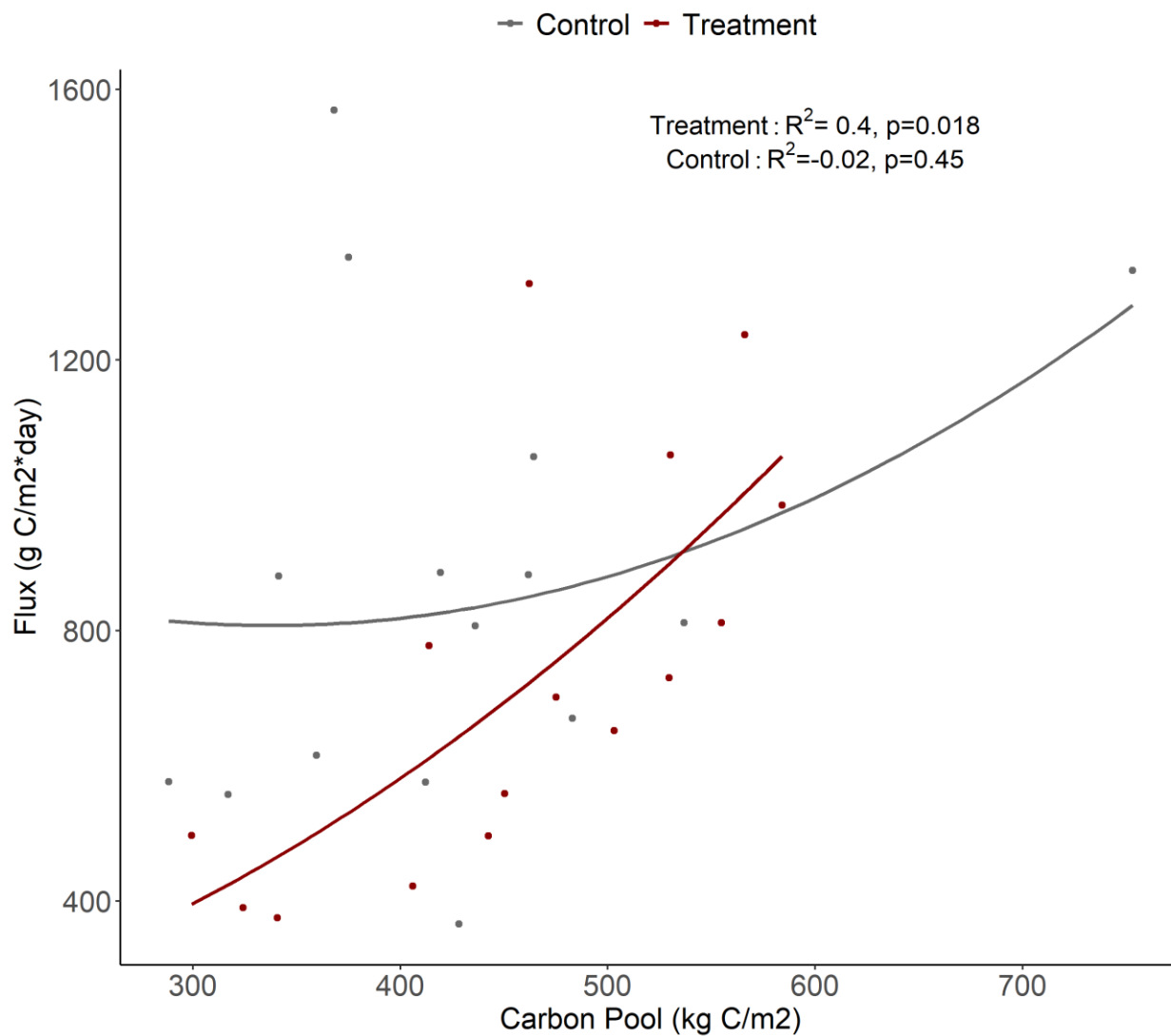| Site		n	Bd	%N	%C	N Pool	C Pool	C:N
PP	C	5	0.76 (0.08)^a	0.8 (0.2)	10.4 (2.7)	38.5 (6.3)	460.4 (76.0)	11.9 (0.1)^{ab}
	T	5	0.61 (0.04)^{ab}	1.1 (0.09)	12.2 (1.05)	47.9 (1.9)	539.4 (20.1)	11.2 (0.08)^a
CH	C	5	0.41 (0.03)^b	0.8 (0.05)	11.9 (1.05)	34.8 (1.9)	467.4 (20.4)	13.4 (0.2)^{bc}
	T	5	0.41 (0.03)^b	0.9 (0.06)	11.5 (0.8)	36.2 (1.8)	462.2 (19.4)	12.7 (0.1)^c
WL	C	5	0.78 (0.04)^a	0.3 (0.02)	4.8 (0.2)	28.2 (2.2)	361.1 (26.7)	12.5 (0.2)^b
	T	3	0.61 (0.04)^{ab}	0.6 (0.01)	8.03 (0.3)	29.5 (2.9)	374.7 (36.6)	12.7 (0.09)^{bc}

Figure 7. Relationship between carbon pool and CO_2 flux between treatments at all sites.
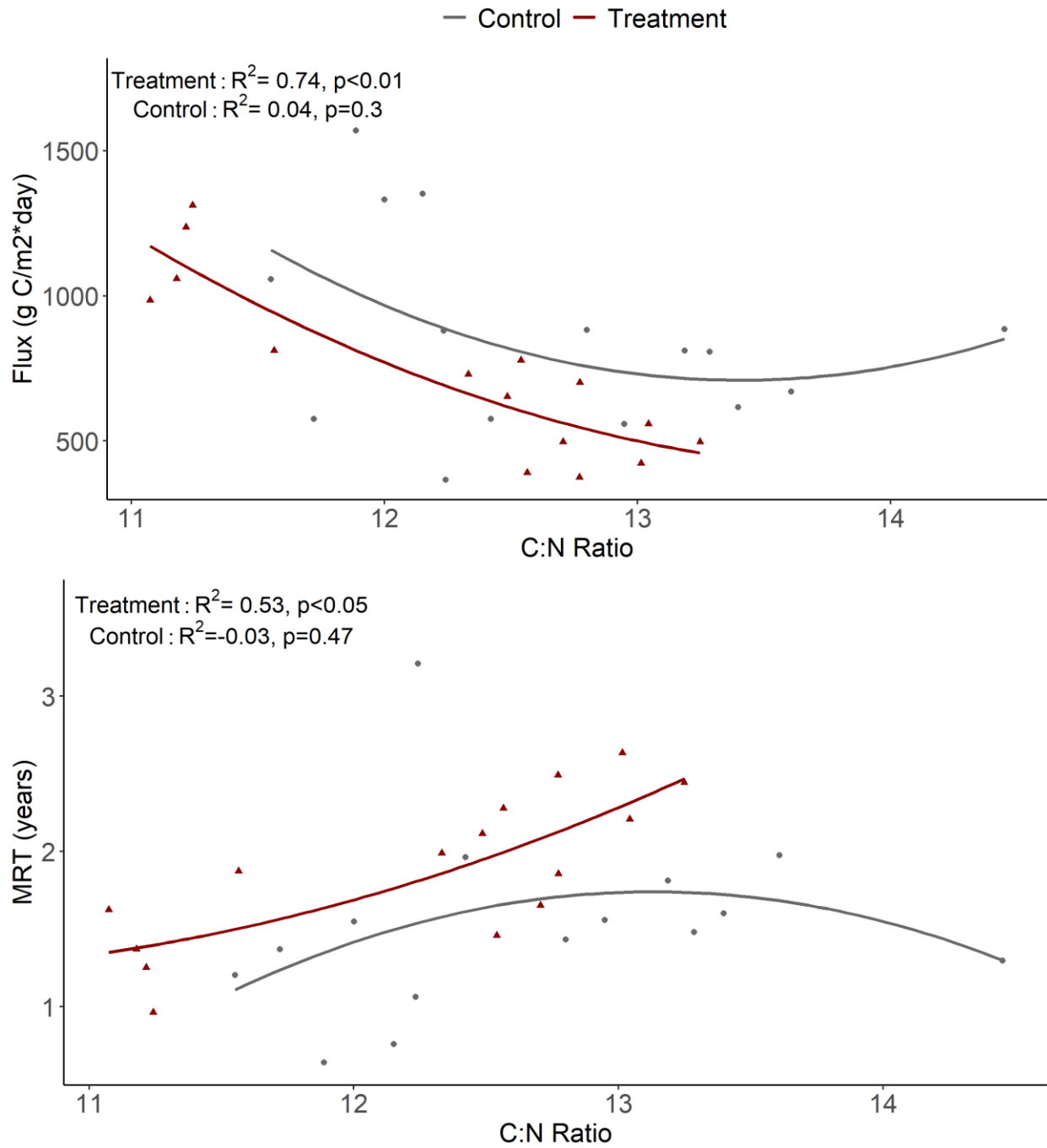
Figure 8. Relationships between the C:N ratio, MRT and CO_2 flux between treatments at all sites.
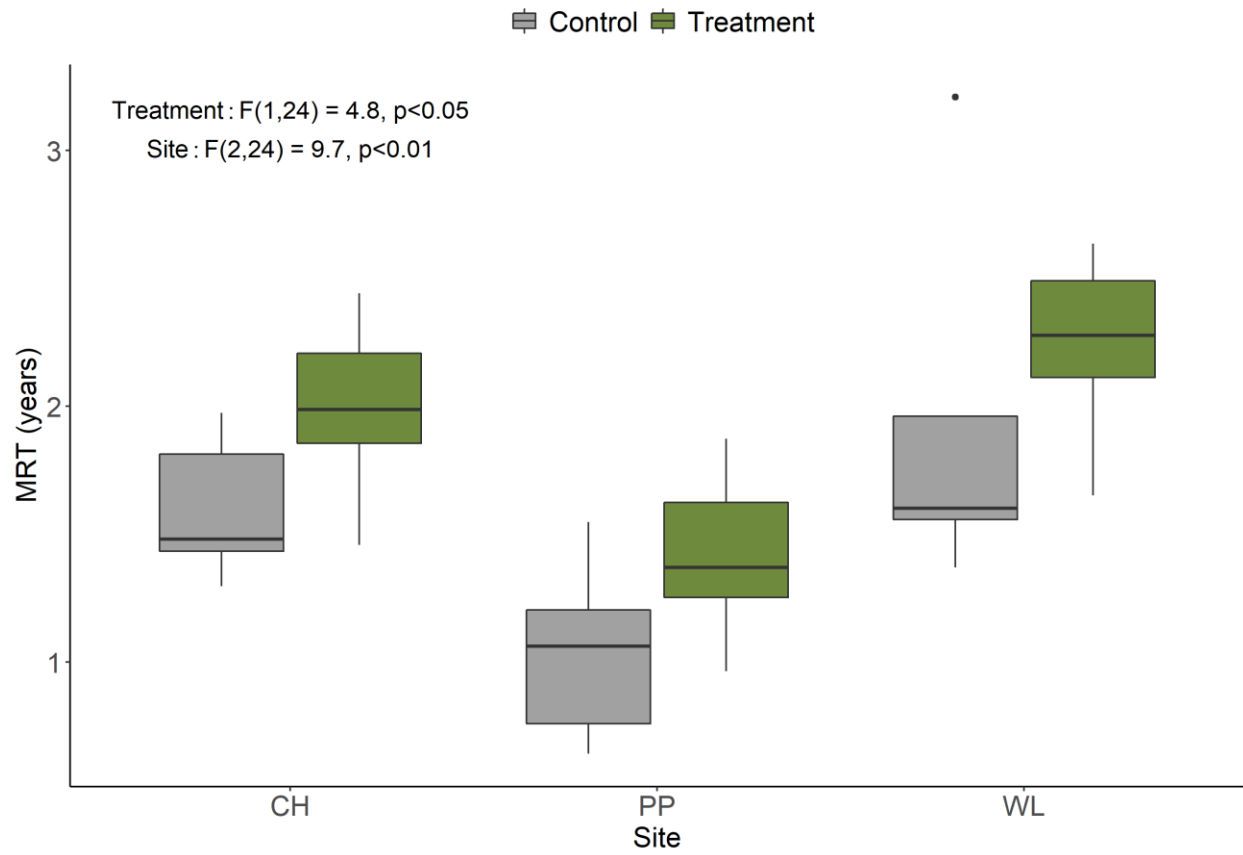
Figure 9. Average (±SE) Mean residence time (MRT) of Soil organic carbon (SOC) for each site and treatment.