THESIS

SOIL HEALTH INDICATORS FOR WATER-LIMITED REGIONS: SENSITIVITY TO COMPOST AND CROPPING INTENSIFICATION

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In partial fulfillment of the requirements
for the Degree of Master of Science
Colorado State University
Fort Collins, Colorado
Summer 2024

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ABSTRACT

SOIL HEALTH INDICATORS FOR WATER-LIMITED REGIONS: SENSITIVITY TO COMPOST AND CROPPING INTENSIFICATION

In the water-limited agroecosystems of the Great Plains, USA, management strategies such as compost application and cropping system intensification have been promoted to increase soil health and help adapt to climatic variability. However, accurately assessing soil health to support production systems in such regions hinges upon a selection of indicators sensitive to management and linked to essential soil functions, especially those related to soil water dynamics. Using a suite of soil physical and biological parameters, this study assessed the effects of management on soil health metrics and evaluated the extent to which these metrics were related to soil water dynamics utilizing long-term studies in Akron, CO, and Clovis, NM. Soil physical indicators included aggregate stability (mean weight diameter; MWD), bulk density and saturated hydraulic conductivity, while biological indicators included measures of soil macrofauna and microbial communities. Compost application was the primary driver of increased aggregate stability and abundance of soil biota at both sites, though effects of cropping system intensification were observed for some indicators. Measures of soil microbial abundance were correlated with MWD, but saturated hydraulic conductivity was generally not correlated with other measured variables. Our findings indicate that MWD and microbial abundance are linked and sensitive to management, and further research to connect measures of soil biological and physical health to soil water dynamics in semi-arid systems is necessary to develop regionally relevant frameworks for soil health assessments.

ACKNOWLEDGEMENTS

I would like to thank my advisors, Meagan Schipanski and Steven Fonte, for their guidance, support and trust through this project. I have grown as a person and as a scientist thanks to their incredible mentorship. Thank you also to Matthew Ross for serving on my committee and proving a new perspective. I would also like to thank our collaborators for all their help: Rajan Ghimire, Prakriti Bista, Sangu Angadi, and Olufemi Adebayo at New Mexico State University, Maysoon Mikha at USDA-ARS Central Great Plains, and Veronica Acosta-Martínez and Jon Cotton at USDA-ARS Cropping Systems Research Laboratory. Additionally, thank you to Ann Hess and the graduate students at the Franklin Graybill Statistical Laboratory for consulting, to Cody Hardy and the crew in Akron, CO, for the maintenance of our plots, and to USDA-NIFA for funding this work.

I have immense gratitude for the CSU Agroecology group who provided invaluable feedback on this research. A special thank you goes to Carolita Landers and Sarah Harper for their aid in the field and in the lab. Lastly, I would like to thank my friends and family for their encouragement and willingness to listen to me talk about soil.

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1. INTRODUCTION

In semi-arid regions, management of agroecosystems under increased temperatures and shifting precipitation patterns due to climate change has presented challenges to producers and scientists alike. Under this context, the imperative to adopt management strategies that balance productivity and sustainability is urgent. Soil health, which is defined as the capacity of soil to function as a living ecosystem, supporting plants, animals and humans (USDA-NRCS, 2022), offers a dynamic ecosystem-level understanding of soil that can be used to guide management decisions and enhance essential overall soil functioning. Assessments of soil health can thus be used to support farmers in adopting more resilient and adaptive management strategies that help to maintain crop productivity and a range of other ecosystem services in the long-term.

Efforts to improve soil health have primarily focused on adoption of management techniques that support four core principles, according to the NRCS: 1) maximize presence of living roots, 2) minimize disturbance, 3) maximize soil cover, and 4) maximize biodiversity (USDA-NRCS, 2022). However, soil health hinges upon complex biophysical and biochemical mechanisms that interact at various temporal and spatial scales and the potential of management practices to enhance soil health is not uniform; a farmer’s ability to improve soil health via management strategies is determined by a host of factors, including sociopolitical and climatic conditions (Gosnell et al., 2019, Prairie et al., 2023, Rosenzweig et al., 2020, Rosenzweig et al., 2018). While increased soil health is not a guarantee of increased yields or profitability (Miner et al., 2020), identifying improved indicators of essential soil functions in regions where those functions are limited due to resource concerns, such as in water or nutrient limitation, could contribute to increased yields and productivity (Foley et al., 2012).

The High Plains aquifer in the Great Plains, USA, supports 30% of total animal and crop production in the country and is worth approximately $35 billion on an annual basis (Hornbeck and Keskin, 2014), however, this critical resource is diminishing across much of the region. Rapid decline of the groundwater levels is forcing thousands of acres of irrigated land to reduce water use, particularly on the western and southern regions of the aquifer. Some wells in this part of the country are predicted to no longer be able to support irrigated agriculture in the next 10-15 years, indicating a large scale conversion to dryland agriculture (Haacker et al., 2016). Managing this transition will be essential to maintaining agricultural communities in this area.

Soil health management strategies suggested to maintain the productivity of agroecosystems in semi-arid regions include implementing intensified rotations with reduced bare fallow periods and/or adding organic amendments such as composted manure (Ghimire et al., 2024). Both strategies increase carbon (C) inputs to the system; compost application does so directly, while intensified rotations do so via increased aboveground residue returns and rhizosphere inputs. The quality and point of entry of C inputs will also likely impact soil health outcomes. For example, surface-applied compost or manure is generally considered higher quality than plant biomass inputs, with lower C:N ratio in manure that can promote microbial communities. However, plant inputs via root exudates and root residues are intimately associated with soil microbial communities and clay-mineral surfaces, supporting their stability (Cotrufo et al. 2013). Compost or manure application in the form of manure and intensified rotations can have positive effects on soil biota, with increases seen in the abundance and diversity of soil microbial communities (Thapa et al. 2021, Liu et al. 2020, Zhen et al. 2014, Ayuke et al. 2011). Additionally, both strategies can benefit soil structure and a range of related soil properties (Liu et al., 2021, Blanco-Canqui et al., 2013). These two management techniques are being rapidly adopted by farmers in the U.S. (USDA-ERS, 2023, USDA-ERS, 2022), but accurately assessing their success in water-limited systems hinges on the responsiveness of the soil health indices to management, and how well those indices relate to essential soil functions (Ghimire et al., 2023).

Existing frameworks for measuring and scoring soil health indicators, including the Soil Management Assessment Framework (SMAF, Wienhold et al., 2009) and the Comprehensive Assessment of Soil Health (CASH, Schindelbeck et al. 2008), have been adapted to manage for geographic variation, such as with the Soil Health Assessment Protocol and Evaluation (SHAPE) tool (Nunes et al., 2021). However, these frameworks are not specifically built for water-limited systems and may not be optimal for contexts where they were not originally developed. These assessment tools rely on threshold values or existing datasets to generate indicator scores, and semi-arid regions are often underrepresented in these datasets (Karlen et al., 2019). Soil organic matter (SOM) is often cited as the primary indicator of soil health in these frameworks (Karlen et al., 2019), and many studies have shown the critical role of SOM in supporting soil health. Increasing SOM to increase water hold capacity and infiltration is commonly recommended (Bhadha et al., 2017), however, in regions such as the Central and Southern Great Plains where SOM content is generally low, creating meaningful increases in SOM as a response to management is often a slow process (Acosta-Martinez et al., 2018). Moreover, considerable uncertainty remains as to the extent of SOM's impact on soil water dynamics (Minasny and McBratney 2017). Subsequently, SOM alone may not be the ideal indicator of soil health in terms of its responsiveness to management in water-limited regions, especially in the short- to medium-term.

Other measures of soil health that play a role in soil water dynamics include measures of soil structure and soil biological communities. Aggregate stability, one aspect of soil structure, is often highly correlated with water infiltration rates and saturated hydraulic conductivity (Basset et al. 2023, Bagnall et al. 2022). Well-aggregated soils create pore spaces for water to move through, increasing water capture during heavy rain events (Franzluebbers 2002), which is of extreme value in dryland systems that rely on highly variable and often intense precipitation events. Wet aggregate stability, often assessed as mean weight diameter (MWD), measures the quantity of the soil that resists slaking and mechanical disturbance during wet-sieving, an important proxy of a soil's resistance to heavy rainfall and erosion. Aggregate stability and associated water dynamics are likely to be mediated, at least in part, by soil biological communities, including microbes (Six et al. 2004, Rillig et al. 2002), soil macrofauna (Deleon et al., 2020, Kelly et al., 2020), and living roots (Thomas et al. 1993). Macroaggregates are held together with binding agents such as roots, hyphae and polysaccharides, while microaggregates form within them and freely in the soil matrix (Lutzow et al., 2006, Six et al., 2002). The formation and turnover of aggregates is also influenced by the presence of soil fungi, with increased aggregation thought to be associated with hyphal enmeshment of soil particles and the production of glomalin by arbuscular mycorrhizal fungi (Morris et al., 2019). Obtaining aggregate stability data in conjunction with information on soil biological communities can provide unique insights on how management influences soil structure and associates processes.

Methods of measuring soil microbiomes are diverse, and selecting one that balances efficiency, accuracy and cost is important to the feasibility of soil health assessments. Ester-linked fatty acid methyl ester (EL-FAME) profiling of microbial communities is a reliable tool for estimation of microbial community size and is sensitive to management changes (Pérez-Guzmán et al. 2023, Cotton et al. 2013), and is correlated with other commonly used methods for characterizing microbial communities, including PLFA (Li et al. 2020) and 16S/ITS amplicon sequencing (Pérez-Guzmán et al. 2023). Furthermore, EL-FAME is a simpler methodology and a lower cost than PLFA (Li et al, 2020), which is of considerable value for repeatable and saleable soil microbial community evaluations. The recent development of the CNPS activity assay, which measures the activities of four major soil enzymes known to cycle C, N, P and S simultaneously (Acosta-Martínez et al. 2019) and has been shown to be highly correlated with EL-FAME profiling (Pérez-Guzmán et al. 2023), could also serve as a useful tool to measure this soil health indicator.

Direct measurements of soil water dynamics, including measures of infiltration rate, time to runoff, or saturated hydraulic conductivity (K_{fs}), are of deep value in assessing the impact of management strategies on water capture and retention in water-limited systems. However, these methods have posed challenges to past soil health assessments due to large variance, requiring a high degree of replication when measured in-field, and a lack of response to management when used with disturbed samples (Bagnall et al. 2022, Bagnall et al. 2023). Moreover, decisions made by researchers regarding study design, the type and quality of tools used, and temporal and spatial scales of assessment influence K_{fs} results (Deb and Shuka, 2012). An automated method to measure K_{fs} in real time could provide a streamlined, user-friendly technique for this measure that reduces labor and human error. Examining large datasets with a host of soil health indicators and assessing them in relation to measures of soil water dynamics will support the development of proxy-indicators of soil water dynamics, which has been suggested to mitigate the aforementioned challenges (Basset et al, 2023).

Our study utilizes long-term study experiments in Colorado and New Mexico to assess the effects of cropping system intensity and compost application on multiple soil health indicators, with a focus on water dynamics in these water-limited systems. Indicators selected included aggregate stability as a measure of soil structure, EL-FAME analysis, CNPS activity and soil macrofauna communities as measures of soil biological activity and diversity, and saturated hydraulic conductivity (K_{fs}) as a measure of soil water dynamics. We also sought to explore relationships between soil health indicators to better understand the potential factors that mediate soil water dynamics. We hypothesized that compost applications and cropping intensification would both enhance soil aggregation, soil biological communities, and saturated hydraulic conductivity. In addition, we hypothesized that increased biological activity and abundance (or biomass) would enhance aggregate stability, thereby increasing saturated hydraulic conductivity rates (Fig. 1).

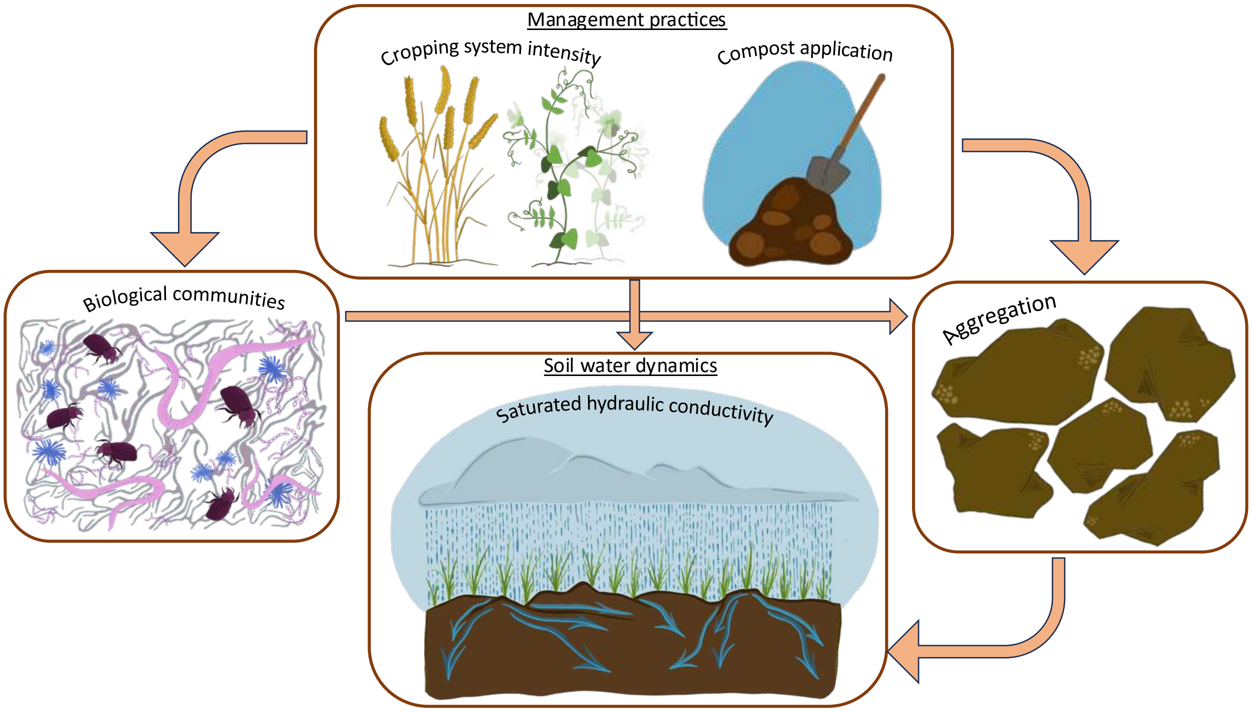

Figure 1. Conceptual model of hypothesized relationships between management practices (cropping system intensity and compost application) and soil health indicators (biological communities, aggregation, and saturated hydraulic conductivity).

2. METHODS

2.1. **Study sites**

Sites were selected at research facilities in New Mexico and Colorado with existing compost and cover cropping trials. The two sites chosen were the USDA-ARS Great Plains field station near Akron, CO (40˚09′ N, 103˚09′ W, elev. 1384 m.) and New Mexico State University Agricultural Science Center in Clovis, NM (34° 35′; 103° 12W′, elev. 1348 m.). In Akron, soils are Weld silt loam (fine, smectitic, mesic Aridic Argiustoll) and Rago silt loam (fine, smectitic, mesic Pachic Argiustoll), classified as Mollisols. Soil in Clovis is an Olton clay loam (fine, mixed, thermic Aridic Paleustol), classified as an Ultisol. Both sites have semiarid climates and are considered water-limited: Clovis experiences an average mean annual precipitation of 470 mm and Akron has an average annual precipitation of 420 mm.

The experiment in Akron, CO was established to assess the impact of compost additions and cropping intensity on dryland system productivity and soil health. Beginning in the fall of 2010, compost beef feedlot manure was applied every 2 years at three levels: the recommended rate for crop N use (1X, 22.9 t ha^{-1}), five-times the recommended rate (5X, 108.7 t ha^{-1}), and no compost (0X). In the spring of 2022, the 5X plots were reduced to the recommended rate, 1X. The recommended rate is based on the N needs of the crop and the anticipated plant available N released in the year after application (Davis and Westfall, 2014). This site existed as an uncultivated grassland with no inputs until it was cultivated in 2010 at the establishment of this experiment. From 2010 to 2022, two 2-year crop rotations existed on the site: a wheat/fallow rotation, and a triticale (*Triticosecale rimpaui*) + forage pea (*Pisum sativum* L.)/fallow. In 2022, the triticale + forage pea/fallow rotation was changed to a wheat/forage pea rotation (with no summer fallow), resulting in two rotations at the site for the duration of this study, varying in cropping intensity: wheat/fallow (W/F), wheat/forage pea (W/P). All treatments were implemented as no-till, but a shallow sweep has been used as needed in recent years (since 2020) as a form of minimum tillage to control herbicide resistant weeds. Further information about the site's history can be found in Calderón et al. (2018). This experiment is arranged as a split-plot experimental design with four replicate blocks, separated by 13.7 m native grass buffer strips. Each replicate block consists of three main plots, one of each of the compost application levels. Each of the compost plots were split into four randomized sub-plots (15.2 x 9 m): two per crop rotation, such that both phases of each rotation were present every season.

The Clovis, NM, experiment was established in October of 2015 as a cover cropping trial in an irrigated wheat-sorghum-based rotation, with fallow or different cover crop treatments grown in year three of the rotation. Within the cover crop phase, there are eight cover crop treatments at this site, however, only three were sampled in this study to cover a range of diversity: 1) a six-species mix (SSM) containing pea, oat (*Avena sativa L.*), canola (*Brassica napus L.*), hairy vetch (*Vicia villosa L.*), forage radish (*Raphanus sativus L.*), and barley (*Hordeum vulgare L.*), 2) a low diversity treatment with just forage pea (*Pisum sativum* L.), and 3) a bare fallow, with no cover crop planted. The study employs a randomized complete block design with three replicates, and each plot is 18 m x 12 m. This site was under conventional tillage management prior to study implementation in October 2015, but has since been managed as a no-till system. Further information on the establishment and design of this study site can be found in Ghimire et al (2019). Cover crops were planted in February, biomass was terminated in May, and residue was retained in the field. Starting in the spring of 2022, compost application began in Clovis on a sub-plot (6m x 6m) within each main plot to establish two compost treatments: 1) the recommended rate for crop N use (1X, 16.8 t ha^{-1}) and no compost (0X, in the rest of the main plot).

These two sites then represent long- and short-term use of the treatments examined in this study: compost application and cropping intensification and their interaction. Akron, with 12 years of compost application and 2 years of cropping intensification, constitutes the long-term compost application and short-term cropping intensification. Clovis, with 7 years of cover cropping and compost application beginning at the start of this study, constitutes the long-term cover cropping/cropping intensification and short-term compost application. Climatic differences existed between the 2022 and 2023 sampling seasons: during 2022, Akron experienced a D2 level severe drought and Clovis experienced a D4 level exceptional drought, however, in 2022 Akron did not experience drought and Clovis experienced a D1 moderate drought (USDA, NDMC, NOAA, 2024).

2.2. **Soil sampling**

In June of 2022 and 2023, soil samples from the 0-10 cm depth were taken at each site. In Akron in 2022, both phases of the W/P rotation were sampled, resulting in 32 samples collected (2 compost levels x 2 crop phases x 2 samples per plot x 4 replicates). In 2023, both phases of the two rotations were sampled, resulting in 64 samples taken from Akron (2 compost levels x 2 rotations x 2 crop phases x 2 samples per plot x 4 replicates). In Clovis in 2022 and 2023, soil samples were collected from the sorghum phase of the rotation resulting in 36 samples (3 cover crop treatments x 2 compost application rates x 3 replicates x 2 sub-samples per plot).

Two sample points were chosen randomly within each plot, each at least 1 m away from the edge. Two soil samples were collected with a square spade to a depth of 10 cm and composited from each sampling point for chemical and microbial analysis. Bulk density was measured at the 0-5 cm and 5-10 cm depths by gently hammering in a sharpened metal cylinder (7 cm dia. x 5 cm deep). Samples were removed from the cylinders, transferred to sealed plastic bags and placed in protective sleeves to prevent compaction. All soil samples were kept in coolers with ice packs during transport to the lab.

2.3. **Soil macrofauna**

Soil macrofauna communities were assessed via excavation and hand-sorting, adapted from the Tropical Soil Biology and Fertility method (Anderson and Ingram, 1994). A soil monolith (25 x 25 cm) was excavated with a flat shovel to a depth of 20 cm. All soil, together with surface residues, was hand sorted, such that all macrofauna visible to the naked eye (> 2 mm) were collected and stored in 70% ethanol and then transported to the lab for identification (generally to the level of order). Total abundance of macrofauna was calculated as the number of specimens per soil monolith and then scaled up to a per m^2 basis. Diversity was calculated as species richness (i.e., total number of taxonomic groups per monolith) and using the Shannon diversity index (Shannon 1948). Due to the high amount of labor involved in this measure and the relatively low number of individuals observed, macrofauna were only collected in 2022.

2.4. **Saturated hydraulic conductivity**

Both sites were assessed for field saturated hydraulic conductivity (K_{fs} cm sec^{-1}) using SATURO automated infiltrometers (Meter Group, Inc.) in the late summer of 2022 and 2023. To measure K_{fs}, which describes the ease of water to pass through pore spaces in soil under field-saturated conditions, SATUROs utilize a simplified version of the two-ponding head approach (Reynolds and Elrick, 1990) that accounts for soil capillarity, depth of ponding ring radius, and depth of ring insertion. For this experiment, the settings used included a 30-min soak time, 5 cm difference in pressure heads, and 2-cycle runs. SATUROs calculate K_{fs} from the final cycle of the run. In 2022, the 5 cm ring insertion depth was used, however, due to leaks and pooling the 10 cm ring insertion depth was used instead in 2023. Each year, 32 SATURO measurements were taken in Akron (2 compost levels x 2 rotations x 2 crop phases x 4 replicates), and 18 were taken in Clovis (2 compost levels x 3 rotations x 3 replicates).

Data was exported, and all runs with standard error larger than the measured K_{fs} value were manually recalculated using the simplified equation from Nimmo et al. (2009), as instructed in the SATURO manual. Runs in which the last cycle did not produce adequate pressure or flux, were also recalculated using the raw data from the first cycle.

2.5. **Soil processing**

In the laboratory, field-moist bulk density cores were weighed. A representative sub-sample (~ 20 g) of soil was weighed and dried in a 60 °C oven, and then reweighed to calculate the gravimetric water content. The remaining soil from the bulk density cores from both depths were then combined, and gently passed through an 8 mm sieve prior to air drying for subsequent analysis of wet aggregate stability. Bulk soil samples were kept field-moist, separated into subsamples, and overnight shipped in coolers for microbial analysis.

2.6. **Aggregate stability**

Using a wet sieving methods adapted from Elliot (1986), 35 g of air-dried, 8-mm sieved soil per sample was separated into four size fractions: 1) large macroaggregates (> 2000 µm), 2) small macroaggregates (250-2000 µm), 3) microaggregates (53-250 µm), and 4) silt and clay (<53 µm). The 8-mm sieved soil was submerged over a 2 mm sieve in deionized water for slaking for 5 min., and then manually sieved by moving the sieve up and down (in and out of the water) for 50 cycles over a period of 2 min. The soil remaining on top of the sieve was washed into pre-weighed aluminum pan, and the soil and water in the pan below was poured over a 250 μm sieve in another pan. The sieving process was then repeated with the 250 μm sieve, and then a 53 μm sieve. The remaining water and soil in the pan after the 53 μm sieving represents the silt and clay fraction, which was also collected into pre-weighed aluminum pans. All fractions were oven-dried at 60°C (typically 1-3 days, depending on the fraction).

The oven-dry soil fractions were weighed, and the weight of the pan was subtracted from the total dry mass to obtain the mass of each size fraction. The mean weight diameter (MWD) of the aggregates was calculated using the average size of each fraction, multiplied by the proportional size of each fraction (% of total subsample), and added together (van Bavel 1950). Rocks and large pieces of plant material were removed from the largest size fraction and subtracted from the total weight when calculating MWD.

2.7**. EL-FAME microbial analysis**

Field moist samples were shipped overnight to the USDA-ARS facility in Lubbock, TX. The composition of the soil microbial community was evaluated using ester-linked fatty acid methyl ester (EL-FAME) profiling in which EL-FAMEs are extracted and analyzed following the protocol described in Li et al (2020). This process provides the relative abundance of soil microorganisms by assessing individual fatty acids and matching them to known biomarkers.

Forty-six named FAME biomarkers were used for identification and quantification. For bacterial biomarkers, gram-positive (i13:0, a13:0, i14:0, a14:0, i15:0, a15:0, i15:1 ω6c, a15:1ω9c, i16:0, a16:0, i17:0, a17:0, i17:1ω9c, i18:0, i19:0), gram-negative (cy17:0ω7c, i17:0 3OH, cy19:0ω7c, 14:1ω9c, 14:1ω8c, 15:1ω9c, 15:1ω8c, 15:1ω6c, 16:1ω9c, 16:1ω7c, 16:0 2OH, 17:1ω8c, cy17:0ω7c, 18:1ω7c, 18:1ω6c, 18:1ω5c, 19:1ω9c, 19:1ω8c, 19:1ω7c), and actinobacteria (10Me16:0, 10Me17:1ω7c, 10Me17:0, 10Me18:1ω7c, 10Me18:0, 10Me19:1ω7c) were used. For fungal biomarkers, saprophytic fungi (18:3ω6c, 18:4ω3c, 18:2ω6c, 18:1ω9c), and arbuscular mycorrhizal fungi (16:1ω5c) were used. Protozoa biomarkers (20:4 ω6c) were also assessed. The absolute quantity of individual FAMEs was expressed as nmol g^{-1} soil. Total fungi were calculated as the sum of AMF and saprophytic fungal biomarkers, and total bacteria was calculated as the sum of gram-negative, gram-positive, and actinobacteria. The fungi to bacteria (F:B) ratio was calculated as the ratio of total fungi to total bacteria.

2.8 **Enzyme activity (CNPS activity)**

Soil enzyme activity (EA) was also measured at the USDA-ARS facility in Lubbock, TX, using an assay that assesses four EAs (β-glucosidase, β-glucosaminidase, acid phosphomonoesterase, and arylsulfatase) simultaneously, and combines the results into a single measurement called "CNPS activity" (Acosta-Martínez et al. 2019). The four EAs used in this assay were chosen for their respective nutrient cycling abilities: β-glucosaminidase for C and N cycling, β-glucosidase for C cycling, acid phosphomonoesterase for P cycling, and arylsulfatase for N cycling. Briefly, air-dried, 4-mm sieved soils were incubated for 1 hour at 37°C in 0.5ml 0.5M acetate buffer and 0.5ml of each of the following substrates: 0.05 M *p*-Nitrophenyl-β-D-glucopyranoside, 0.01 M *p*-Nitrophenyl-N-acetyl-β-D-glucosaminide, 0.05 M *p*-Nitrophenyl phosphate, and 0.05 M *p*-Nitrophenyl sulfate. After, 0.5 ml of 1.0 M $CaCl_2$ and 2 ml of 0.1 M THAM buffer were added and the suspended soil was filtered, releasing *p*-nitrophenol (PNP). PNP was then determined with a visible spectrophotometer (Thermo Scientific Evolution 60S) colorimetrically at 400 nm.

2.9 **Data Analysis**

The measured soil properties were compared using mixed effects ANOVA. In Akron, compost, rotation, phase, and all possible interactions were included as fixed effects, while block, compost plot, and sub-plot were included as a random effect to address the split-plot design. In Clovis, compost, crop rotation and their interaction were used as fixed effects, while block, rotational plot, and sub-plot were included as random effects. Sub-plot as a random effect was removed from the ANOVA design for K_{fs} at both sites because only one measurement was taken per plot. Estimated marginal means were calculated and pairwise comparisons were conducted using the Tukey correction. Simple linear regression was used to analyze the relationships between soil aggregate stability, soil fungal FAMEs, CNPS activity, and saturated hydraulic conductivity using 2023 data. Between-class principal component analysis (PCA) was also conducted on data from 2023, and significance of treatment groups was assessed with Monte-Carlo permutation tests. Log or square root transformations were used where necessary to meet model assumptions, and significance was defined as a p value <0.05. One data point in the Akron 2023 EL-FAME dataset was removed as an unexplained outlier. All data analysis was conducted in R version 4.3.1 (R Core Team, 2023).

3. RESULTS

3.1. **Soil physical characteristics**

Soil physical properties in Akron, CO, were strongly impacted by compost application and by phase to a lesser extent, however, they showed little impact of rotation. Bulk density (BD) was reduced by compost additions in both years, and in 2023 fallow phases displayed increased BD as compared to phases with wheat or forage pea (Table 2). Aggregate stability followed a similar trend in Akron: in 2022, MWD was increased by compost (Table 1), while in 2023 it was affected by both compost application and phase (Fig. 2). Composted plots demonstrated 102% greater MWD (610 μm) than un-composted plots (302 μm), and plots with live roots at the time of sampling (i.e., the wheat or forage pea phases of either rotation) averaged a 24% greater MWD (479 μm) than plots under fallow (387 μm). There were no effects of cropping system or significant interactions between compost and cropping system treatments on MWD in Akron. Saturated hydraulic conductivity (K_{fs}) results from Akron displayed positive effect of compost on K_{fs} in both years, as well as an effect of phase seen in 2023 with the highest K_{fs} rates seen in the wheat phase of the W/F rotation (Fig. 3).

In Clovis, NM, soil physical properties were affected by different treatments in each year. In 2022, BD was not affected by treatments, however, in 2023 an interaction between compost and rotation was found on BD, with the forage pea treatment without compost applied having the highest BD (Table 2). In Clovis, no effects of cropping system or compost application were observed on MWD in 2023 (Fig. 2), however, a moderately significant ($p = 0.059$) positive effect of compost application was observed on MWD in 2022, with composted plots displaying 19% greater MWD (Table 1). K_{fs} results in Clovis differed between the two years: in 2022, K_{fs} showed a significant effect of rotation ($p = 0.002$) and an interaction between rotation and compost (p = 0.046) with the Forage Pea treatment having the highest K_{fs} values and compost-amended Forage Pea plots displaying increased variability of K_{fs} as compared to the un-composted plots. In 2023 K_{fs} was affected by compost application, with composted plots having a higher K_{fs} than un-composted (Fig. 3).

Table 1: Soil physical and biological properties in: a) Akron, CO and b) Clovis, NM, from 2022. In Akron, measurements were collected under both phases (Wheat, Forage Pea) of the W/P rotation at both compost application levels (0x corresponding to no compost applied, 5x corresponding to 108.7 t ha^{-1}; n = 32). In Clovis, data was collected in all rotations (Fallow, Forage Pea, SSM), and under both compost application levels (0x corresponding to no compost applied, 1x corresponding to 16.8 t ha^{-11}; n = 32). Average values are the mean ± SD.

a) Akron, CO, 2022									
Rotation	**Phase**	**Compost**	**Bulk Density (g cm^{-3})**	**Mean Weight Diameter (μu) ***	**Total FAMEs ***	**Total bacteria ***	**Total fungi ***	**Fungi : Bacteria ***	**CNPS activity (mg PNP kg^{-1} soil h^{-1})**
W/P	Forage Pea	0x	1.12 ± 0.07	285 ± 31	235 ± 29.6	69.4 ± 8.35	61.1 ± 8.42	0.88 ± 0.08	283 ± 61.3
		5x	0.99 ± 0.09	608 ± 154	772 ± 341	180 ± 66.9	247 ± 77.6	1.39 ± 0.13	334 ± 42.0
	Wheat	0x	1.09 ± 0.08	336 ± 103	317 ± 211	70.7 ± 10.4	65.7 ± 8.16	0.94 ± 0.11	248 ± 71.6
		5x	0.99 ± 0.07	703 ± 173	719 ± 85.43	150 ± 11.6	202 ± 32.4	1.33 ± 0.12	301 ± 37.8
ANOVA									
Compost			0.057	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.002**	0.135
Phase			0.577	0.114	0.421	0.443	0.470	0.750	0.121
Compost x Phase			0.558	0.940	0.477	0.337	0.100	0.075	0.968

b) Clovis, NM, 2022								
Rotation	**Compost**	**Bulk Density (g cm^{-3})**	**Mean Weight Diameter (μu)**	**Total FAMEs ***	**Total bacteria ***	**Total Fungi ***	**Fungi: Bacteria ***	**CNPS activity (mg PNP kg^{-1} soil h^{-1})**
Fallow	0x	1.39 ± 0.05	211 ± 28	124 ± 17.4	41.0 ± 6.41	35.7 ± 4.73	0.88 ± 0.06	195 ± 55.6
	1x	1.41 ± 0.06	308 ± 82	138 ± 17.1	45.5 ± 8.66	41.4 ± 12.8	0.9 ± 0.1	180 ± 43.7
Pea	0x	1.39 ± 0.07	252 ± 44	141 ± 26.4	40.8 ± 6.70	35.2 ± 8.27	0.86 ± 0.08	216 ± 40.0
	1x	1.30 ± 0.07	274 ± 112	147 ± 36.2	42.7 ± 9.83	39.6 ± 11.38	0.92 ± 0.07	192 ± 48.1
SSM	0x	1.35 ± 0.05	304 ± 99	148 ± 29.0	44.0 ± 8.58	43.0 ± 8.18	0.98 ± 0.05	218 ± 58.4
	1x	1.35 ± 0.08	328 ± 56	170 ± 31.3	48.7 ± 6.45	44.4 ± 7.80	0.91 ± 0.07	222 ± 40.70
ANOVA								
Compost		0.282	0.059	0.092	0.172	0.224	0.897	0.480
Rotation		0.076	0.220	0.259	0.352	0.225	0.150	0.274
Compost x Rotation		0.095	0.356	0.740	0.892	0.845	0.133	0.765

Table 2: Soil physical and biological properties in a) Akron, CO and a) Clovis, NM, from 2023. In Akron, data was collected in all phases of both the Wheat/Fallow (W/F) and Wheat/Forage Pea (W/P) rotations under both compost application levels (0x corresponding to no compost applied, 5x corresponding to 108.7 t ha^{-1}), n = 64. In Clovis, data was collected from the sorghum phase of all rotations (Fallow, Forage Pea, SSM), and both compost application levels (0x corresponding to no compost applied, 1x corresponding to 16.8 t ha^{-11}), n = 36. Average values are the mean ± SD.

a) Akron, CO, 2023							
Rotation	**Phase**	**Compost**	**Bulk Density (g cm^{-3})**	**Total FAMEs**	**Total bacteria (nmol g^{-1} soil)**	**Fungi: Bacteria**	**CNPS activity (mg PNP kg^{-1} soil h^{-1})**

				(nmol g^{-1} soil) *			
W/F	Fallow	0x	1.14 ± 0.11	189 ± 27.1	60.13 ± 9.74	0.75 ± 0.08	305 ± 105
		5x	1.00 ± 0.07	617 ± 135	138.13 ± 25.58	1.08 ± 0.12	432 ± 128
	Wheat	0x	1.01 ± 0.21	229 ± 59.8	79.80 ± 15.64	0.82 ± 0.14	411 ± 115
		5x	0.87 ± 0.12	756 ± 168	151.78 ± 29.51	1.38 ± 0.12	539 ± 55.5
W/P	Forage Pea	0x	1.10 ± 0.03	229 ± 28.8	65.59 ± 8.07	0.93 ± 0.11	265 ± 112
		5x	0.94 ± 0.07	703 ± 114	149.73 ± 21.45	1.24 ± 0.13	427 ± 76.2
	Wheat	0x	1.13 ± 0.11	267 ± 51.7	78.31 ± 10.88	0.79 ± 0.08	356 ± 38.5
		5x	0.98 ± 0.06	647 ± 98.1	137.3 ± 19.74	1.27 ± 0.09	418 ± 81.2
				ANOVA			
Compost			**0.007**	**<0.001**	**<0.001**	**<0.001**	0.054
Rotation			0.502	0.427	0.960	0.102	**0.019**
Phase			0.196	**0.001**	0.104	**0.036**	**0.003**
Compost x Rotation			0.821	0.399	0.738	0.461	0.746
Compost x Phase			0.562	**0.040**	0.131	**0.002**	0.260
Rotation x Phase			**0.013**	**0.008**	0.110	**0.001**	0.140
Compost x Rotation x Phase			0.449	0.791	0.350	0.631	0.267

b) Clovis, NM, 2023

Rotation	**Compost**	**Bulk Density (g cm^{-3})**	**Total FAMEs (nmol g^{-1} soil)**	**Total bacteria (nmol g^{-1} soil)**	**Fungi: Bacteria**	**CNPS activity (mg PNP kg^{-1} soil h^{-1})**
Fallow	0x	1.26 ± 0.03	155 ± 41.6	46.4 ± 11.2	0.88 ± 0.09	282 ± 79.2
	1x	1.20 ± 0.02	240 ± 40.4	67.9 ± 12.0	0.94 ± 0.13	337 ± 84.5
Pea	0x	1.73 ± 0.02	245 ± 60.2	65.77 ± 10.98	1.03 ± 0.17	390 ± 168
	1x	1.19 ± 0.04	242 ± 41.0	67.0 ± 10.2	1 ± 0.08	374 ± 164
SSM	0x	1.20 ± 0.04	214 ± 30.8	61.0 ± 8.29	0.99 ± 0.08	405 ± 86.4
	1x	1.21 ± 0.04	243 ± 63.1	67.7 ± 14.0	1.01 ± 0.1	399 ± 101
			ANOVA			
Compost		0.280	**0.023**	**0.010**	0.523	0.778
Rotation		0.110	0.192	0.301	**0.006**	0.153
Compost x Rotation		**0.035**	0.078	0.069	0.394	0.740

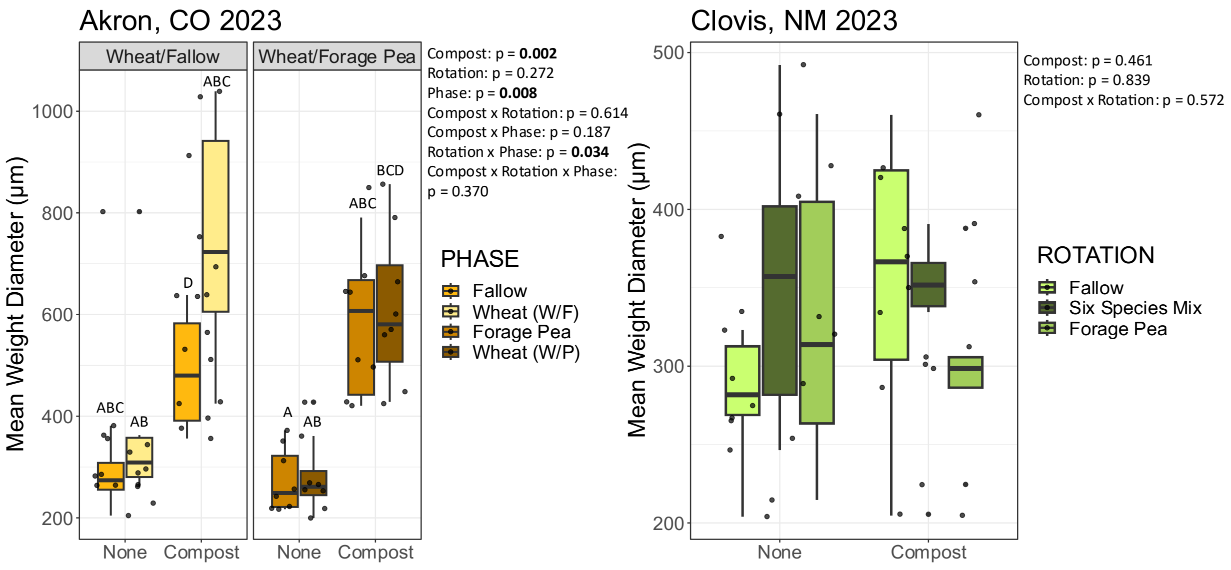

Figure 2. Aggregate stability, expressed as mean weight diameter (MWD), under different compost treatments in all phases of both rotations examined (W/F, W/P) in Akron, CO, and under different compost treatments and rotations (Fallow, Forage Pea, Six Species Mix) in Clovis, NM, from samples collected in June of 2023. Different letters represent the pairwise comparisons of estimated marginal means.

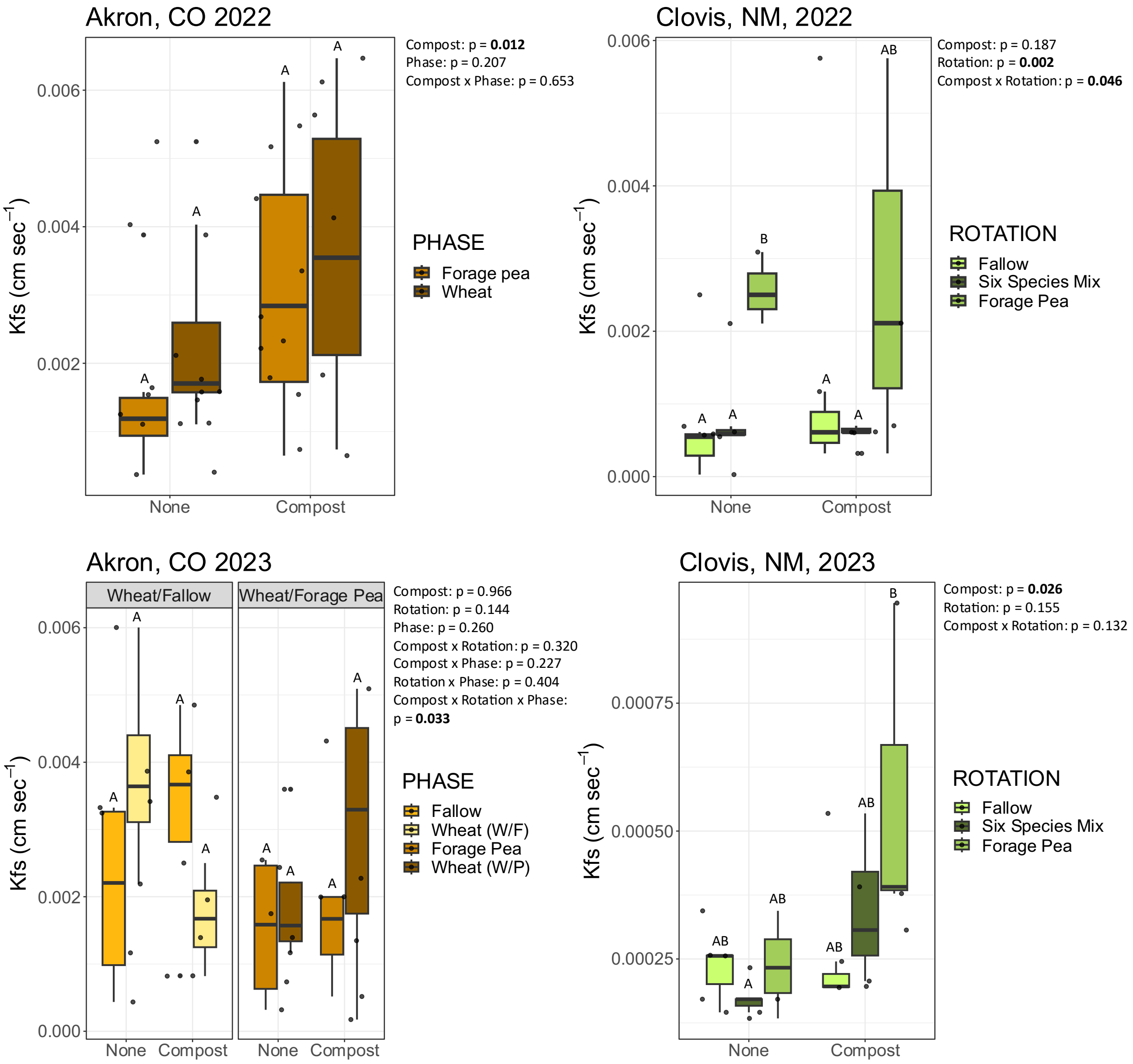

Figure 3. Saturated hydraulic conductivity (Kfs, cm sec^{-1}) in Akron, CO, and Clovis, NM, in 2022 and 2023. In Akron in 2022, Kfs under both compost treatments and both phases of the W/P rotation are shown (n = 32). In Akron in 2023, Kfs under both compost treatments and all phases of both the W/F and W/P rotations are shown (n = 32). In Clovis for 2022 and 2023, Kfs under both compost treatments and all rotations are shown (n = 36). Different letters represent the pairwise comparisons of estimated marginal means.

3.2 **Soil biological communities**

3.2.1 Soil macrofauna

Soil macrofauna communities examined in 2022 showed small differences between treatments. In Akron, effects of phase were found on total abundance of all macrofauna, as well as on total beetles, with wheat having the highest abundance across species, and forage pea having the highest abundance of beetles (Table 3). In Clovis, short-term compost application had a more nuanced effect, with a significant interaction indicating that total macrofauna abundance and beetle abundance increased with compost amendment under forage pea but not for the other rotations (Table 3). No significant effects were observed for the Shannon diversity index or total earthworm abundance at either site.

Table 3. Macrofauna abundance, species richness, Shannon diversity index, total earthworms and total beetles from Akron, CO, and Clovis, NM, in 2022. Data from both phases of the W/P rotation and both compost levels in Akron and from all rotations and both compost levels in Clovis is shown. Average values are the mean ± SD. An asterisk indicates that the variable was square root transformed for ANOVA.

Akron, CO, 2022							
Rotation	**Phase**	**Compost**	**Total abundance (Ind. m^{-2}) ***	**Species richness**	**Shannon diversity index**	**Earthworms (Ind. m^{-2})**	**Beetles (Ind. m^{-2})**
W/P	Wheat	0x	50 ± 26	1.5	0.296	2 ± 6	6 ± 8
		5x	114 ± 132	2.375	0.654	0 ± 0	82 ± 90
	Forage Pea	0x	30 ± 18	1.5	0.37	10 ± 15	18 ± 20
		5x	114 ± 132	2.675	0.613	2 ± 6	64 ± 49
ANOVA							
Compost			0.907	0.129	0.230	0.101	0.873
Phase			**0.014**	0.766	0.926	0.101	**0.003**
Compost x Phase			0.509	0.766	0.749	0.318	0.426

Clovis, NM, 2022						
Rotation	**Compost**	**Total abundance (Ind. m^{-2}) ***	**Species richness**	**Shannon diversity index**	**Earthworms (Ind. m^{-2})**	**Beetles (Ind. m^{-2})**
Fallow	0x	147 ± 88	3	0.834	77 ± 97	16 ± 20
	1x	123 ± 120	3.5	0.985	53 ± 108	13 ± 15
Pea	0x	93 ± 74	2.667	0.667	37 ± 47	11 ± 19
	1x	328 ± 179	4.833	1.164	133 ± 140	51 ± 53
SSM	0x	144 ± 36	4.333	1.303	32 ± 44	27 ± 22
	1x	104 ± 39	3	0.935	43 ± 56	8 ± 13
ANOVA						
Compost		0.165	0.462	0.609	0.352	0.501
Rotation		0.190	0.761	0.564	0.542	0.322
Compost x Rotation		**0.008**	0.089	0.179	0.259	**0.037**

3.2.2. EL-FAME microbial analysis

Compost inputs and cropping system intensification (rotation) resulted in clear differences in microbial communities, measured via EL-FAME analysis, at both sites and in both years. In Akron, compost increased all biomarkers examined in both years ($p < 0.001$ for all taxa; Table 1 and 2). In 2023, composted plots averaged a 198% greater quantity of total FAMEs than un-composted plots (680 nmol g^{-1} soil and 228.38 nmol g^{-1} soil, respectively; Table 2). This difference is even greater when examining only fungal FAMEs (Fig. 4), with composted plots averaging 210% greater quantity of fungal biomarkers than un-composted plots. Effects of phase where also observed in Akron in 2023 on total FAME biomarkers, fungal FAME biomarkers, and on the F:B ratio (Table 2, Fig. 4), with differences observed between phases under the no compost treatment, while no effects of phase were observed in 2022 (Table 1). A significant interaction between compost and phase and between rotation and phase was also observed on the F:B ratio in 2023, such that compost generally increased the F:B ratios, but wheat phases of the W/F rotation with or without compost had the highest F:B ratios (Table 2).

In Clovis, effects of compost application were observed on total FAME biomarker abundance, total bacteria, and total fungi in 2023 (Table 2, Fig. 4), with composted plots averaging an 18% greater quantity of total FAME biomarkers than un-compost plots (242 nmol/g soil and 205 nmol/g soil, respectively; Table 2). In 2022, compost had a moderately significant ($p = 0.092$) effect on total FAMEs (Table 1). Effects of rotation were observed on the F:B ratio (Table 2, Fig. 4), but no rotation effect was found on fungal or bacterial biomarkers alone. However, 30% more fungal FAME biomarkers were observed in 2023 under the forage pea rotation as compared to the fallow (67.4 nmol g^{-1} soil and 52.0 nmol g^{-1} soil, respectively: Table 2), and 4.4% more were found under forage pea as compared to SSM (67.4 nmol g^{-1} soil and 64.6 nmol g^{-1} soil, respectively; Table 2). No effect of rotation was observed on any FAME biomarkers in 2022.

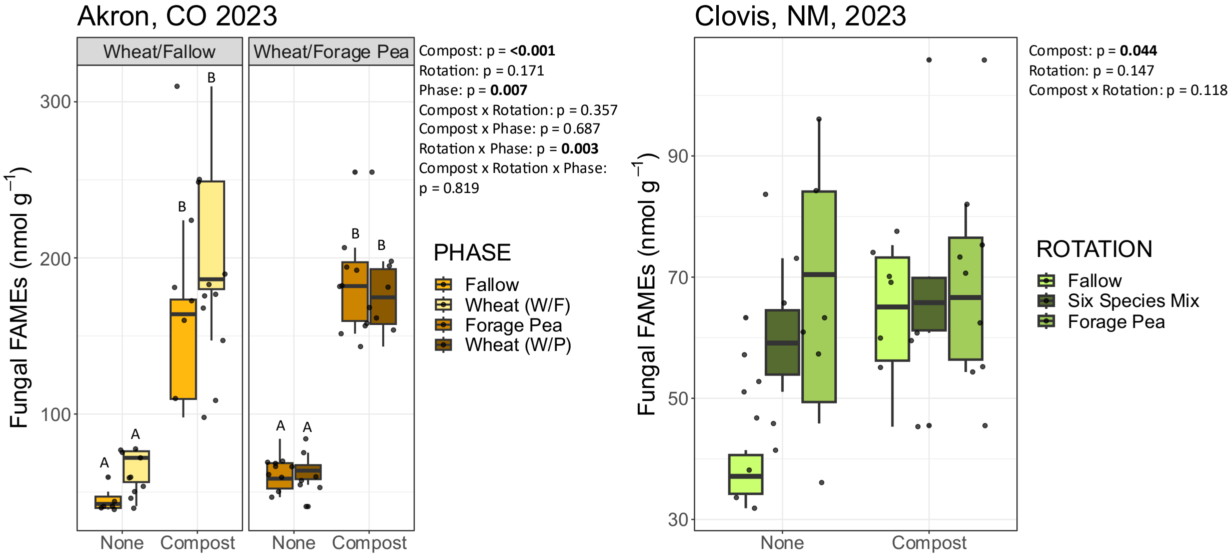

Figure 4. Total abundance of fungal fatty acid methyl easter biomarkers (FAMEs, nmol g^{-1} soil) for all fungal tax examined in Akron, CO, and Clovis, NM, in 2023. In Akron, fungal FAMEs were log transformed, under all phases of both the W/F and W/P rotations, under both compost application levels (0x corresponding to no compost applied, 5x corresponding to 108.7 t ha^{-1}) is shown. In Clovis, fungal FAMEs under wheat, pea, and six-species mix (SSM) rotations, under both compost application levels (0x corresponding to no compost applied, 1x corresponding to 16.8 t ha^{-1}) is shown. Different letters represent the pairwise comparisons of estimated marginal means.

3.2.3. Enzyme activity (CNPS activity)

Soil enzyme activity (EA), measured as "CNPS activity" with a combined assay for four EAs known to cycle soil C, N, P and S (β-glucosidase, β-glucosaminidase, acid phosphomonoesterase, and arylsulfatase), was highly sensitive to treatments in Akron, CO, but not in Clovis, NM. In Akron, CNPS activity was moderately significantly affected by compost application ($p = 0.054$), and significantly affected by rotation and phase of the rotation in 2023 (Table 2). The wheat phase of the W/F rotation displayed increased CNPS activity compared to the fallow phase, and the W/F rotation had increased variability between phases and compost treatments as compared to the W/P rotation. The forage pea phase of the W/P moderately increased CNPS activity compared to the wheat phase in composted plots, however, the opposite was true in the un-composted plots. CNPS activity in Akron was not affected by treatments in 2022. In Clovis, no effects of treatments were observed on CNPS activity in 2023 or in 2022.

3.4. **Exploring relationships between soil health indicators**

To test our hypothesized relationships between variables (Fig 1), we explored simple linear relationships between indicators and conducted principal component analyses (PCAs) for each site using 2023 data. In Akron, significant positive correlations were found between fungal FAMEs and MWD ($R^2 = 0.491$, $p < 0.001$), between CNPS activity and MWD ($R^2 = 0.309$, $p < 0.001$), and between CNPS activity and fungal FAMEs ($R^2 = 0.381$, $p < 0.001$), however, no correlation was found between MWD and K_{fs} ($R^2 = 0.007$, $p = 0.658$). Results of PCAs from Akron (Fig. 6) largely validated results from univariate methods of analysis, providing clear differentiation between compost treatments ($p = 0.001$, Fig. 5) along PC1, but there was no difference between rotation phases. PC1 explained 57.5% of the variability in the dataset and was associated with microbial indicators (fungi, bacteria, CNPS activity) and aggregate stability (MWD) on one side, and bulk density projecting in the opposite direction. PC2, which explained 18.4% of the variability, was primarily associated with saturated hydraulic conductivity (K_{fs}).

In Clovis, we found similar positive correlations between fungal FAMEs and MWD ($R^2 = 0.296$, $p < 0.001$), between CNPS activity and MWD ($R^2 = 0.226$, $p < 0.001$), and between CNPS activity and fungal FAMEs ($R^2 = 0.318$, $p < 0.001$), though the strength of the relationships was weaker than at Akron. Again, no correlation was found between MWD and K_{fs} ($R^2 = 0.007$, $p = 0.658$), however, a significant positive correlation was found between fungal FAMEs and K_{fs} ($R^2 = 0.288$, $p = 0.022$) in Clovis. PCAs with data from Clovis (Fig. 5) did not show differentiation between treatments when assessed by compost or by rotation. PC1, which explained 55% of the variability, was primarily defined by microbial indicators (fungi and bacteria) and PC2, which explained 19.4% of the variability, was primarily associated with aggregate stability (MWD).

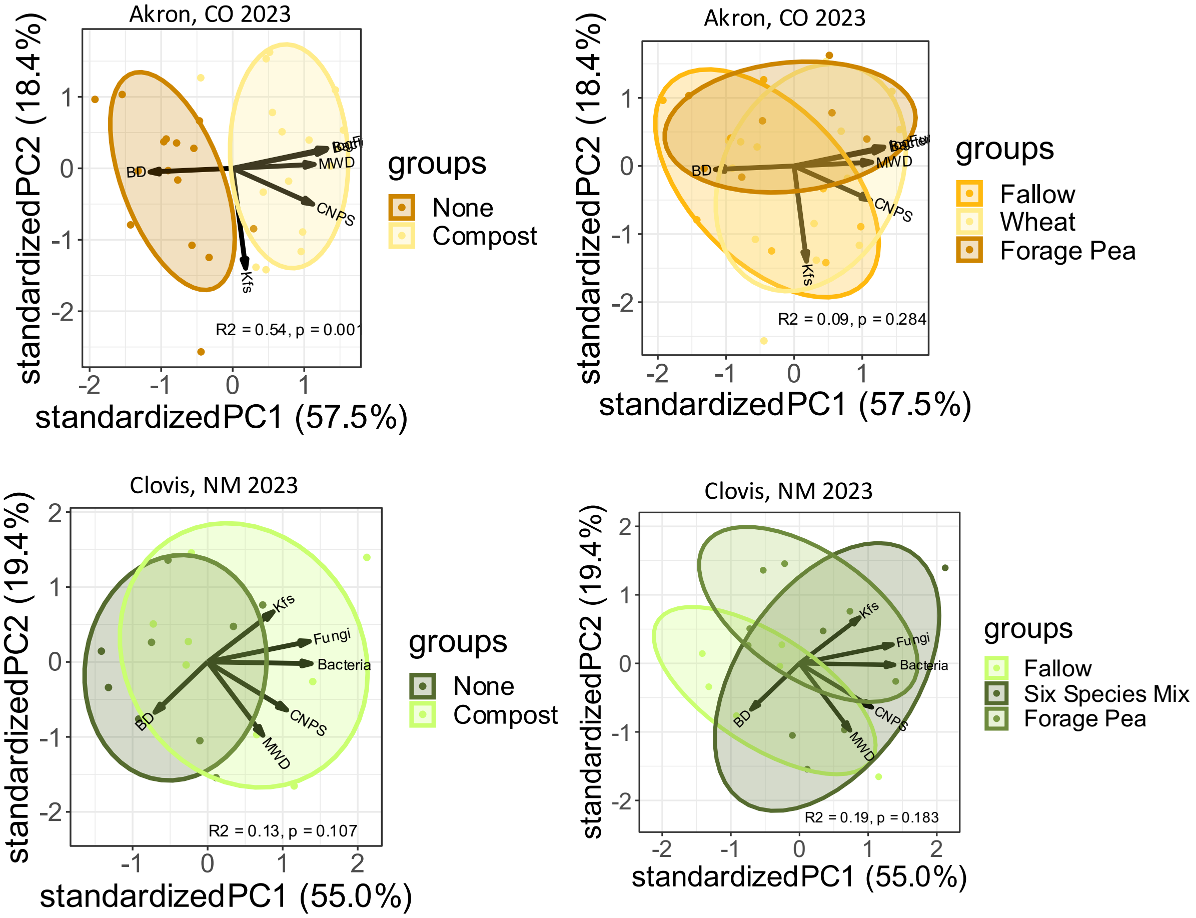

Figure 5. Between-class analysis of compost treatments (left) and rotation treatments (right) via principal component analysis (PCA) from Akron (top) and Clovis (bottom) with data from 2023. Ordination diagram displays the sample data in relation to soil health indicators: fungi, bacteria, enzyme activity (CNPS), mean weight diameter (MWD), bulk density (BD), and saturated hydraulic conductivity (Kfs). In Akron, principal components 1 and 2 explain 57.5% and 18.4% of the variation, respectively. In Clovis, principal components 1 and 2 explain 55% and 19.4% of the variation, respectively.

4. DISCUSSION

4.1. **Impacts of compost management**

Composted manure application was the primary driver of changes in observed physical and biological soil health indicators at both sites. Inputs of organic matter (OM) via manure application have been shown to increase water stable aggregate formation after long- and short-term use (Whalen et al., 2003, Aoyama et al., 1999), which was evidenced in this study by significant increases in soil aggregation in Akron, the long-term compost application site, and in 2022 in Clovis, the short-term compost application site. (Fig. 2, Table 1). Previous research from the same experimental plots in Akron obtained similar findings in which the 5X compost treatment had 40% greater MWD than the 0X plots (Liu et al. 2021). Compost application as a form of nutrient inputs can increase wheat yields in semi-arid regions (Calderón et al. 2018, Reeve et al. 2012), and thus has the potential to affect aggregate stability by increasing crop productivity and residue return to soil, thereby increasing overall OM inputs to the system (Campbell et al. 2001). Compost inputs also increased saturated hydraulic conductivity, but the effect was inconsistent between the two sites and years. The positive effect of compost in Akron that was found in 2022 but not 2023 may have been due to difference in ring depth of the SATURO between the years or interannual climate variability, as 2022 was a drier year than 2023, resulting in a more pronounced effect of compost on K_{fs}. Effects of compost on soil water dynamics were also found in Clovis in 2023, however, increased K_{fs} rates were only found in the composted intensified rotations and not in the fallow rotation, suggesting a varying impact of compost.

Increased OM inputs also increased the size and activity of soil biological communities. Application of composted manure can increase microbial abundance and diversity (Zhen et al., 2014), microbial biomass C and N (Ren et al. 2019), and enzyme activity (Liu et al., 2020), resulting in functional changes to microbial metabolic cycling of C, N and P (Liu et al., 2022). The findings from this study were in alignment with past research, with compost application resulting in higher quantities of total FAME biomarkers at both sites (Table 1 & 2), as well as higher CNPS activity in Akron (Fig. 5).

Impacts of organic amendments on soil macrofauna are often positive, particularly on those that feed on SOM and can be considered “ecosystem engineers”, such as earthworms. Compost application can stimulate earthworm activity (Nare et al., 2017, Sharpley et al., 2011), abundance, and diversity (Ayuke et al., 2011), however, most studies have been conducted in wetter climates. We did not find a stimulating effect of compost on abundance of earthworms or other ecosystem engineers. Earthworm activity is typically the greatest during wet and warm times of the year, so these results may be due in part to climatic conditions during the first year of data collection for the study, as both sites experienced a pronounced drought in 2022 (USDA, NDMC, NOAA, 2024).

Results from this study suggest a generally positive effect of composted manure application on soil health indicators in water-limited systems. Though reliance on animal manure use for nutrient management present challenges, including low nutrient content per unit weight, variability in nutrient concentrations (Schoenau and Davis, 2006), and the potential for salt accumulation and over-application of phosphorus relative to N (Gondek et al. 2020), the benefits of this type of amendment in water-limited systems are strong. Increased aggregation and abundance of soil biota, as found in this study and in previous work, are indicative of the benefit of this practice on soil health. The use of animal manure in the Central and Southern Great Plains is particularly relevant due to the regional proximity of animal-farming operations and local availability of manure.

4.2. **Impacts of cropping system**

While not as pronounced as compost effects, cropping system and phase of the rotation influenced multiple soil health parameters. In Akron, where all phases of the high-intensity (W/P) and the low-intensity (W/F) rotations were sampled in 2023, the only soil health indicator sensitive to the intensity of the rotation was soil enzyme activity (Table 2), though other physical (BD, MWD) and biological (total FAMEs, Fungi, and F:B) indicators were sensitive to the phase at the time of sampling and some displayed a significant interaction between rotation and phase. In particular, the presence of living roots at the time of sampling (i.e.: wheat or forage pea phases of either rotation) contributed to lower bulk density and higher levels of aggregation and microbial abundance and activity as compared to phases without living roots (i.e.: the fallow phase). This trend was also present, but weaker at Clovis, in which rotations with increased intensity and year-round living roots had higher F:B ratios, higher levels of CNPS activity, and higher K_{fs} rates in 2022 ($p<0.1$). Previous research has found that living roots in the soil influence soil physical and biological characteristics, evidenced by impacts on soil structure and aggregation (Fonte et al., 2019, Materechera et al., 1992, Reid and Gos, 1982), soil water movement (Rasse et al., 2000), and soil biology (Bodegom et al., 2018, Xing-Feng et al 2014). The influence of living roots on biology is of particular interest as root exudates are known to stimulate microbial activity and abundance by providing C to the rhizosphere (Herms et al., 2022, Walker et al., 2003) and triggering the “rhizosphere effect” (Hartmann et al, 2008), attracting microbes to roots, and initiating cross-spatial communication between root and microbe (Jain et al., 2020, Hirsch et al., 2013, Walker et al 2003). These past findings are in alignment with effects found in Akron of phase on microbial indicators. The relatively pronounced effect of phase also indicates that these variables change seasonally, and that this should be accounted for in future soil health evaluations.

Intensified dryland rotations can also increase annualized crop residue inputs, up to double that of a summer-fallow system (Peterson and Westfall, 2004). If residue is retained in the system, increased cropping system intensity subsequently leads to increased organic C return to the system (Engel et al., 2017, Sherrod et al., 2003) and increased physical protection of soil from wind and water erosion (Blanco-Canqui and Wortmann, 2017, Hammerbeck et al., 2012). Previous work has also found increased aggregate stability associated with increased C inputs (Kong et al., 2005) and with increased ground cover (Mulumba and Lal, 2008). Although C inputs and ground cover were not measured in this study, a previous study at the Akron experimental site found that C inputs (from compost or residue) resulted in increased total soil C, and that those inputs were positively related to MWD (Liu et al., 2021). Those results were mirrored in this study, during which the intensive cropping systems in Akron significantly increased MWD in 2023 (Fig. 2) and moderately increased MWD in Clovis in 2022, suggesting an effect of residue retention and presence of living roots/rhizosphere inputs on soil structure.

Increased cropping system intensity is often achieved by growing an increased number of different crops or forages, meaning that intensified systems are often more diverse. Prior research has found that increased plant diversity, separately from intensity of the cropping system, can increase microbial biomass and F:B ratios (Lange et al., 2014, McDaniel et al., 2014). Results from this research displayed higher F:B ratios in intensified, diversified treatments (Table 1), indicating transitions toward fungal-dominated microbiomes. Furthermore, the highest diversity treatment in Clovis (six-species mix) tended to have higher levels of soil enzyme activity than other treatments. It is possible that the diversity of the intensified rotations in this study drove changes found in microbial communities, however, determining the cause of these results as increased intensification or diversification is challenging as the two to go hand in hand.

4.3. **Effectiveness of indicators for soil health frameworks**

While the selected soil health indicators can be used to assess the differences that resulted from management practices, they can also be used to quantify the functional relationships between indicators. Soil physical characteristics are mediated by biological communities, and biological communities are supported by soil physical traits (Six et al., 2004, Oades 1993). We hypothesized that soil water dynamics would be mediated by the interaction between soil biological and physical characteristics. Our hypothesis was partially supported as we found correlations between microbial indicators (Fungi, Bacteria, and CNPS activity) and soil structure (MWD) at both sites (Fig. 6), suggesting an influence of microbes on aggregation or vice versa.

Increased OM inputs, whether directly from compost application or via residue return, are likely supporting soil microbial communities and the production of associated by-products that can act as "glue", adhering soil particles together and increasing aggregate stability (Six et al., 2004). For example, glomalin, a glycoprotein produced by AM fungi that can act as this "glue", has been found to be a primary driver of increases in aggregate MWD in some research (Rillig et al., 2002), while others suggest found fungal hyphal length and/or roots to be drivers of aggregation through enmeshment of soil particles (Thomas et al., 1993, Bearden and Peterson, 2000). Additionally, fungal communities are expected to slow macroaggregate turnover and enhance the persistence of SOM in agricultural soils (Six et al., 2004). Results from this study indicated significant relationships between MWD and fungal FAME biomarkers in Akron ($R^2 = 0.491$) and Clovis ($R^2 = 0.296$), which aligns with previous findings and suggests a positive impact of soil fungi on aggregate stability (Rosenzweig et al., 2018). Consistent and abundant OM additions in Akron could result in confounding effects, in which compost is increasing aggregation by itself while also stimulating soil biological activities that increase aggregation.

The influence of soil biota on structure has been studied extensively, however, the effect of biota and structure on functional soil properties, like soil water dynamics, is not as well documented in *in-situ* studies. Improving soil water functions, such as drought tolerance and water holding capacity, via improved soil health has been promoted in semi-arid agroecosystems, however, linking soil health indicators to soil water functions has proven challenging. By evaluating correlations between structural/biological measurements and soil water dynamic measurements we have the potential to determine ideal proxies for indicators that are challenging to measure, such as water infiltration, retention, and movement in soil (Basset et al., 2023, Bagnall et al., 2022). In this study, SATURO Automated Field Infiltrometers were chosen to measure K_{fs} for their potential ease of use, however, SATURO measurements were time-consuming (120 min. per sample), and results were highly variable. Though past work has found MWD to be a predictor of K_{fs} (Basset et al., 2023), no consistent correlations were found between K_{fs} and soil structural indicators, or between K_{fs} and biological indicators in this study. Results from this study indicate that, if K_{fs} measurement via SATURO is used in future research, study designs should consider allocating additional resources to sampling to ensure sufficient replication for addressing the inherent variability of the measurement and challenges associated with the method.

K_{fs}, which assesses the ease by which water can move through pore spaces in soil under field saturated conditions (Childs and Collis-George, 1950), is only one aspect of soil water dynamics. Other measures, including infiltration rate, time to runoff, and soil water storage, also determine soil water dynamics. These factors, though not measured in this study, are likely of high importance in water-limited agroecosystems because they measure the ability of the soil to capture and retain water from precipitation events. Previous studies have found infiltration rate (initial, mean or steady state) to be sensitive to changes in management and to have a positive response to strategies such as organic amendments, reduction of tillage, and cropping system management (Basset et al., 2023, Basche and DeLonge, 2019). Measures such as infiltration rate are of particular interest in this region under the context of climate change, as precipitation in the Central and Southern Great Plains in expected to become more variable and temperature in expected to increase (Kunkel et al., 2014), a context which furthers the necessity of accurate and repeatable soil water dynamic measurements to assess management suggestions.

4.4. **Potential of indicators for use in soil health assessments**

With biological and physical soil health assessments, including but not limited to aggregate stability, soil microbial and macrofaunal communities, and measures (direct or proxy) of soil water dynamics, we studied how different management practices influenced a range of potential agroecosystem functions. Selecting and expanding the use of accurate and repeatable methods for measuring soil biological and physical health is essential to building frameworks for soil health assessments in water-limited regions, and to determining best management practices for a climatically variable future. Employing a group of common and agreed-upon soil health indicators for use in future regional- and national- scale soil health tests will allow for the creation of larger datasets without the concern of the impact of differences in methodology (Bagnall et al., 2023). Regional differences, however, should also be considered with the use of a “minimum suite” of indicators, as challenges and goals of agricultural systems will vary by climate region (Bagnall et al., 2023). In regions such as the Central and Southern Great Plains where water is often a limiting factor, management strategies focused on increasing soil water functions should be a primary goal.

As interest in scalable soil health assessments rises and become more widely available, measuring soil health in relation and response to management will likely become a driving force in the soil science field. Additionally, these assessments will play a key role in greenhouse gas mitigation efforts, including incentivized efforts for C sequestration and subsequent use in C markets (Paustian et al., 2016, Rejesus et al., 2021), and will also support programs such as Colorado's Saving Tomorrow's Agricultural Resources, "S.T.A.R", program which scores and rewards farmers who employ practices that raise soil health. Being able to accurately measure and compare results will determine the success of such programs, while also allowing for regionally specific targets and indicators.

5. CONCLUSIONS

In summary, this study analyzed the impact of compost application and cropping intensity on soil physical and biological indicators to assess the sensitivity of indicators to management and to quantify the relationships between indicators in water-limited systems. Our results indicated that compost was the main driver of changes to soil health indicators. Cropping system also impacted some indicators, but phase at the time of sampling had a stronger effect than the entire rotation, indicating seasonal effects of rotation on soil health indicators. Positive relationships between aggregate stability and microbial indicators, including fungi and enzyme activity, were found at both study sites. Saturated hydraulic conductivity measurements were not correlated with other soil health indicators and were highly variable between sites and years. Other methods and measures of soil water function may be better correlated with indicators of soil biological and physical health. Further research to find proxy-indicators of soil water dynamics is necessary to develop soil health assessment frameworks that are specific to water-limited systems. Future research for soil health testing should consider methods that are scalable, sensitive to management, and related to soil-water functions to support the needs of production in semi-arid regions.

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