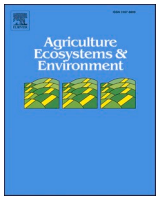
Agriculture, Ecosystems and Environment 393 (2025) 109817

Contents lists available at ScienceDirect

Agriculture, Ecosystems and Environment

journal homepage: www.elsevier.com/locate/agee



Cover crop effects on select soil physicochemical and biological properties

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ARTICLE INFO

Keywords:
Microbial biomass composition
Microbial ratios
Phospholipid fatty acids

ABSTRACT

Land management practices can influence soil physicochemical properties, and these properties regulate microbial community composition and diversity. The objective of this study was to determine the effects of cover crops (CCs) on soil physicochemical and biological properties over 3 years. This study was conducted in a Rhodic Paleudalf using a split-split plot experimental design with vegetative management (CCs vs. no cover crop [NC]) as the whole plot factor. The study used a multi-species mix of CCs including barley (*Hordeum vulgare* L.), flax (*Linum usitassimum*), triticale (*Triticale hexaploide* Lart.), winter wheat (*Triticum aestivum* L.), oats (*Avena sativa*), winter peas (*Lathyrus hirsutus* L.), hairy vetch (*Vicia villosa* Roth.), and crimson clover (*Trifolium incarnatum* L.). Soil samples were collected during 2021 and 2023 at 0–10, 10–20, and 20–30 cm soil depths and analyzed for soil physical, chemical, and biological properties. After three years, soil organic carbon (SOC), volumetric water content (Θ), total N, P, and Mg were 54 %, 17 %, 118 %, 210 %, and 28 % greater, respectively, under CC compared with NC management. These CC-linked improvements in soil properties resulted in significantly greater soil microbial biomass (22 %), total phospholipid fatty acids (8 %), arbuscular mycorrhizal fungi (34 %), and fungi (22 %) than NC management. Over time, CC usage improved nutrient cycling and microbial diversity, while also causing a shift from aerobic to anaerobic microorganisms by increasing Θ. Finally, CCs improved measured soil health indicators in addition to enhancing indicators of soil resilience and these benefits persisted over 3 years.

1. Introduction

The need to provide food, feed, and fiber for a rapidly growing global population, amid the accelerated degradation of finite productive soil, poses a critical challenge that compels governments to develop responsive policies, producers to adapt their practices, and scientists to offer advisory guidance grounded in tested scalable scientific solutions (Prosekov and Ivanova., 2016). As a result, several governments have formulated policies, through incentives and regulations, aimed at improving conservation practices (de Graaff et al., 2013; van Leeuwen et al., 2019). For example, the U.S government, through its Farm Service Agency arm of the Natural Resources Conservation Service offers conservation loans to producers for the implementation of practices directed towards reducing soil erosion and improving soil and water quality. Producers then use these loans to offset some of the costs of implementing conservation practices recommended by scientists. One of the conservation practices is the re-introduction of cover crops (CCs) into crop rotation cycles.

Cover crops have been used in crop production since antiquity, mostly to fortify the soil with nutrients through symbiotic fixation (Kaye and Quemada., 2017). However, CC usage declined in the mid-twentieth century due to the increased availability of commercially produced fertilizers. While synthetic fertilizers provide nutrients in readily available mineral form for immediate plant uptake, this form of soil nutrients are also very mobile and more susceptible to leaching or runoff losses (Grohskopf et al., 2020) and are a major source of water and soil quality degradation under excessive use (Tripathi et al., 2020). On the other hand, symbiotically fixed nutrients in their organic form are less mobile, more environmentally friendly, and provide similar crop yields and quality as their synthetic counterparts (O'Brien and Hatfield., 2019). As a result, the inclusion of CCs in crop rotation practices saw a resurgence in the late twentieth century, and adoption continues to increase in different regions of the world. Cover crops, used as soil primers or commodity crops, can provide several physical, chemical and biological benefits to the soil that can improve soil quality and resilience (Adetunji et al., 2020; Haruna et al., 2020). Some of these benefits are discussed

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https://doi.org/10.1016/j.agee.2025.109817
Received 17 December 2024; Received in revised form 16 May 2025; Accepted 13 June 2025
Available online 26 June 2025

below.

One meta-analysis of 40 different studies with 181 observations by McClelland et al. (2021) revealed a 12 % greater soil organic carbon (SOC) content at the 0–30 cm depth under CC management than with no cover crop (NC). management. Similarly, at the same depth, Villamil et al. (2006) and Haruna et al. (2017) reported increases in SOC of 9 % and 26 %, respectively, compared with NC management. These SOC benefits derive from both above- and belowground CC biomass additions. However, regardless of the CC biomass available, Di Rauso Simeone et al., (2020) reported no noticeable difference in SOC content between CC and NC management. Further, a meta-analysis of 37 studies by Chaplot and Smit (2023) reported mixed results on the effects of CCs on SOC. One possible reason for these inconsistencies could be the growing window, since the SOC provided is significantly greater with continuous CCs than with overwintering and summer CCs (McClelland et al., 2021).

Increases in SOC due to CC management can significantly lower soil bulk density (BD), as reported by several researchers. For example, at the top 10 cm soil depth, CCs have been shown to lower soil BD by 24 % (Nascente et al., 2015), 12 % (Haruna, 2019), 3 % (Adeli et al., 2020), and 18 % (Haque et al., 2024) compared with NC management. Consequently, volumetric water content (Θ) is significantly greater following CC management than NC management due to increased soil porosity (Villamil et al., 2006; Cercioglu et al., 2018) and reduced soil water evaporation (Blanco-Canqui et al., 2011). However, Krstić et al. (2018) reported that, under rain-fed systems, CC treatment significantly reduced Θ due to their evapotranspirational demands. Similarly, a 436 paper meta-analysis found that CCs can lead to lower Θ and groundwater recharge due to higher evapotranspiration than fallow plots (Meyer et al., 2019).

While the effects of CCs on Θ is still currently unclear, Θ drives several soil functions including nutrient availability and microbial activity. The effects of CCs on nutrient availability depend, in part, on the species of CCs used, their nutrient ratios, and soil pH (Maltais-Landry, 2015). Legume CCs have been reported to significantly increase soil N and crop yield compared with NC management due to symbiotic N fixation (Blanco-Canqui et al., 2012). Conversely, non-legume CCs can scavenge extra N in the soil at the end of the growing season. This end-of-season N scavenging is reported to have an environmental (nutrient loading into streams and eutrophication), rather than agronomic (crop production) benefit (Abdalla et al., 2019). Maltais-Landry (2015) demonstrated a greater ability of legume CCs than non-legume CCs to influence rhizosphere properties (e.g., the production of organic acids), and can slightly acidify the soil. However, regardless of the CC species, Adetunji et al. (2021) reported that the presence of CCs had no noticeable effect on soil pH. For soil phosphates (P), Maltais-Landry (2015) reported that CC had no noticeable effect on soil P compared with fallow. However, Soltangheisi et al. (2018) and Hallama et al. (2019) reported that CCs can significantly increase soil P through symbiosis and reduction in particulate loss. The P increases under CC management due to microbial mediation suggests that this practice may also influence microbial composition, activity, and diversity.

Previous studies have shown that soil microbial biomass composition and ratio are greatly regulated by soil physicochemical properties such as Θ, soil temperature, soil pH, and nutrient availability (Peregrina et al., 2014; Yang et al., 2017; Muhammad et al., 2019; Muhammad et al., 2021). By influencing these physical and chemical soil properties, CCs also reportedly affect microbial biomass composition. For example, Njeru et al. (2014), and Brennan and Acosta-Martinez (2017) reported significantly greater arbuscular mycorrhizal fungi (AMF) populations and microbial biomass carbon under CC management than NC management. In their meta-analysis of 81 studies, Muhammad et al. (2021) reported 15 % greater total bacterial populations and 19 % greater total fungal populations under CC than under NC management. However, the same authors (Muhammad et al., 2021) reported that these differences were not significant in mixed-species CC studies. By contrast, Thapa et al. (2021) reported a 31 % larger total microbial community size and a 41 % larger fungal community using mixed-species of CC management than using NC management.

While the effects of single species CC on microbial biomass composition are well documented, results remain conflicting regarding how multi-species CCs affect soil microorganisms (Haruna, 2024). The current shift away from single species towards multi-species CC usage, especially in humid, temperate subtropical climates (Chapagain et al., 2020; upanič and Kramberger., 2023), has created a need to investigate microbial response to multi-species CC usage. More importantly, a need also exists for more studies on the effects of CCs on soil physicochemical properties and microbial biomass composition and ratios in humid, temperate subtropical climates like those of Tennessee with active multi-species CC usage. Over a three-year period, it is hypothesized that 1) due to increased water transpiration by the CC plants, Θ will be significantly lower under CC than NCC management, 2) the coupled symbiotic nutrient addition and scavenging capabilities of the various CC species utilized in this study will result in no net change in soil nutrients between both management practices, and 3) lower Θ and similar soil nutrients between both management practices may increase competition and reduce microbial biomass population and composition over time. The objectives of the present study were to determine 1) water and nutrient availability under CC management, 2) how CCs influence microbial biomass and composition, and 3) the effects of CCs on biotic and abiotic soil health indicators over a three-year study period.

2. Materials and methods

2.1. Site description

The study site was a farmer field located in Murfreesboro, Tennessee, USA (35.816 N, −86.373 W) at an elevation of 190 m above sea level and a 0–2 % slopes (Fig. 1). The soils were classified (USDA taxonomy) as Cumberland silt loam (Rhodic Paleudalfs) (Soil Survey Staff, NRCS, USDA, 2012). Data for soil texture relative to soil depth are presented in Table 1. The 30-year annual temperature and precipitation averages at the study site were 14.6 °C and 1357 mm, respectively. The highest and lowest temperatures during the year occurs during August (32.3 °C) and January (-3.7 °C), respectively. Further, the highest and lowest precipitation occur during the months of May (139 mm) and October (85 mm) of each year.

2.2. Management description

After the harvest of the cash crop (corn, *Zea mays*), the current research field was delineated during September 2020. Prior to that time, the field was managed under 5 years of CC and more than 15 years of no-till management. The present study used a split-split plot design with completely randomized whole plots, with 3 replicates of the whole plots. Each plot was 20.1 m long and 7.1 m wide. There were 2 CC treatments; CC vs. NC, both under no-till management. Based on the current shift from single to multi-species CCs within the region, and the diversity of the CC plant root density and exudates (Haruna, 2024), a mixture of 8 different CCs was used for this study. The CC plants included barley (*Hordeum vulgare* L.), flax (*Linum usitassimum*), triticale (*Triticale hexaploide* Lart.), winter wheat (*Triticum aestivum* L.), oats (*Avena sativa*), winter peas (*Lathyrus hirsutus* L.), hairy vetch (*Vicia villosa* Roth.), and crimson clover (*Trifolium incarnatum* L.). The seeds were first broadcast and then drilled in at the following rates as recommended by the University of Tennessee Cooperative Extension: 15.3 kg ha^{-1} for barley, 50.4 kg ha^{-1} for flax, 22.4 kg ha^{-1} for triticale and winter wheat, 29.1 kg ha^{-1} for oats, 14.6 kg ha^{-1} for winter peas, 5.6 kg ha^{-1} for hairy vetch, and 5.9 kg ha^{-1} for crimson clover. All plots remained under no-till management during this study.

The CCs were planted in October of each year (2020, 2021, and 2022) and terminated in April of the next year using a 4.15 kg ha^{-1} acid

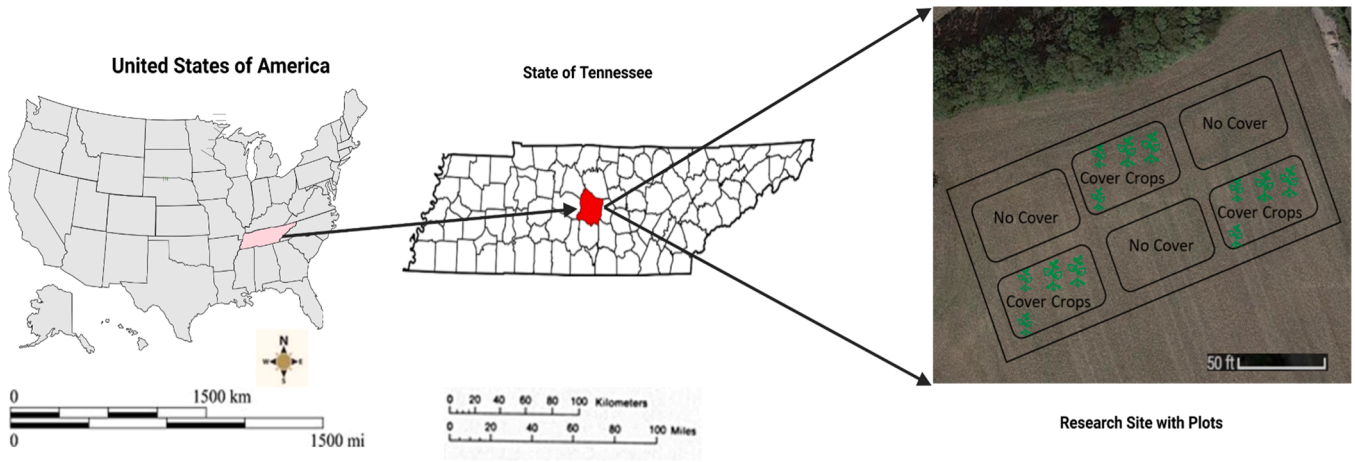

Fig. 1. Study site in Tennessee showing research plots.

equivalent of glyphosate. The termination was completed using two passes of a 9.1 m roller crimp a few hours after glyphosate spraying. The total CC biomass at termination was 1050 kg ha^{-1} during 2021 and 1167 kg ha^{-1} during 2023. The weeds under NC were managed using glyphosate. The commodity crop was corn, planted in April, and harvested in September of each year. All plots received approximately 168 kg ha^{-1} N in the form of urea, 110 kg ha^{-1} P (as P_2O_5), and 114 kg ha^{-1} K (as K_2O) at the start of the commodity crop growing season (based on University of Tennessee Cooperative Extension recommendation and yield goals). After the corn harvest, no extra fertilizers were applied. For further details regarding the study site, please see Haruna et al. (2023), Haque et al. (2024) and Lieskamp et al. (2024).

2.3. Soil sampling

Two sets of soil samples were collected from each plot 1–2 days prior to CC termination during April of 2021 and 2023. The first set of samples for analysis of soil physical and chemical properties was collected using a cylindrical core measuring 143 cm at 0–10, 10–20, and 20–30 cm soil depths. A total of 18 samples (2 treatments × 3 depths × 3 replicates) were collected each year. Excess soil was removed from the top and bottom of the core using a soil spatula. The soils were placed in prelabeled plastic bags and transported to the laboratory for determination of wet soil weight. After weighing, the soils were stored in a refrigerator at 4 °C prior to further laboratory analysis.

A second set of samples were also collected, simultaneously from each plot and at the depths mentioned in the foregoing using a hand auger. In total, another 18 samples were collected each year. This second set of soil samples were used for the determination of microbial biomass and composition. Once collected, the soil samples were placed in prelabeled plastic bags, transported to the laboratory and stored in a refrigerator at 0 °C prior to analysis.

2.4. Analysis of soil physical and chemical properties

For soil physical properties, the first set of samples (soil cores) were removed from the refrigerator and the plastic bags, and dried in an oven at 105 °C until they reached a constant weight (after about 48 hrs). The soil samples were then weighed, and the soil BD was determined using the core method (Grossman and Reinsch, 2002). The gravimetric water content was determined by subtracting the dry soil weight from the wet soil weight. The volumetric water content (Θ) was determined from the gravimetric water content using the BD data. Each soil sample was later ground, passed through a 2 mm diameter sieve, and approximately 10 g of the < 2 mm diameter soil particles were used for particle size analysis using the pipette method (Gee and Or., 2002). Another 10 g of the soil sample was mixed with water at a 1:1 ratio (v/v) and used for pH determination using a standard laboratory pH meter (Peters et al., 2015). Further, approximately 250 mg of the < 2 mm diameter soil particles was used for SOC and total nitrogen (TN) determination by dry combustion in a Skalar® SNC analyzer (Skalar Analytical B.V., Breda, The Netherlands) (Peters and Haruna, 2024b).

Soil nutrients were analyzed by first extracting the nutrients from the soil samples. Nutrient extraction was conducted using 3 g of the < 2 mm soil particle mixed with Mehlich-3 extractant at a soil:extractant ratio of 1:5 (v/v). Soil nutrients analyzed in this study included phosphate (P_2O_5, henceforth referred to as P), magnesium (Mg), potassium (K), and calcium (Ca) contents. This analysis was conducted based on colorimetric methods in a Skalar BluVision® analyzer (Skalar Analytical B.V., Breda, The Netherlands).

2.5. Microbial analysis

Microbial analysis was conducted on the second set of samples collected using a hand auger. These samples were removed from the refrigerator, freeze-dried in a Home Pro freeze-dryer (HarvestRight, Salt Lake City, Utah) for about 48 hrs, and then sent to the University of Missouri Soil Health Assessment Center for analysis. At that soil health assessment center, the lipids were first extracted (using a Bligh-Dyer extractant:chloroform ratio of 4:1 [v/v]) and then segmented in a 96-well (each well was composed of 50 mg of silica) solid phase extraction plate (Phenomenex, Torrence, CA). The lipids were analyzed using a gas chromatograph system (Agilent Technologies, Willmington, DE) controlled by MIS Sherlock™ (ver 6.3. MIDI Corp, Newark, NJ) and Agilent ChemStation software. Further details about this methodology can be found in Buyer and Sasser (2012).

In order to determine the treatment effects on soil microbial community composition, phospholipid fatty acid (PLFA) analysis was used as opposed to PCR-based methods or community level physiological profiling (CLIP). This is because the PLFA analysis is more accurate at differentiating differences in biological markers compared to PCR and CLIP-based methods. For example, in a meta-analysis of 32 studies, Ramsey et al. (2006) reported that PLFA analysis performed accurately at differentiating all biomarkers differentiated by CLIP and PCR-based methods. However, in 44 % of the studies, PLFA analysis performed better at differentiating markers not resolved by CLIP analysis (Ramsey et al., 2006). Also, in 20 % of the studies analyzed by Ramsey et al. (2006), PLFA analysis performed better at differentiating markers not resolved by PCR-based methods. Additionally, PLFA analysis are easier to interpret than amino sugars analysis (Liang et al., 2008).

The fatty acids (and their chemical groups) from the phospholipid fatty acid (PLFA) analysis used as biomarkers for microbial biomass are

Table 1
Particle size distribution with soil depth for the study site (Cumberland silt loam).

	Silt	Sand	Clay
	%		
Depth (cm)			
0–10	64.17	23.33	12.50
10–20	62.50	21.67	15.83
20–30	60.83	20.83	18.33

shown in Table 2. For a complete list of all PFLAs that can be identified by the MIDI software, please see Norris et al. (2023). To avoid redundancy, please note that subcategories were not included in the sum of the entire category. For example, arbuscular mycorrhizal fungi (AMF) are fungi, but the AMF markers were not included in the fungi category.

For this study, the calculated microbial ratios included fungi:bacteria (F:B), predator:prey (Pred:Prey), gram-positive:gram-negative bacteria (Gr+:Gr-), saturated:unsaturated PLFAs (sat:unsat), monounsaturated: polyunsaturated PLFAs (mono:poly), gram-metabolic status:stress (status:stress), monounsaturated fatty acids (MUFA):branched PLFAs (MUFA:branched). The Gr- and Gr+ bacteria were also designated as prey, while eukaryote were designated as predators.

Under normal environmental conditions, large amounts of MUFAs (e.g., 16:1ω7) are produced by Gr- bacteria during metabolism. However, environmental and nutritional stresses promote the conversion of most of the unsaturated fatty acid components into cyclo fatty acids, such as 17:0 cyclopropane and 19:0 cyclopropane. This results in a slowdown of metabolic activity and cell division. Therefore, the methods of Villanueva et al. (2004) and Kaur et al. (2005) can be used to calculate an index of stress as follows:

$$Stress = \frac{(17:0cyclo + 19:0cyclo)}{(16:1\omega 7c + 18:1\omega 7c)} \quad (1)$$

where 17:0cyclo and 19:0cyclo are fatty acids produced by Gr- bacteria under stressful conditions during metabolism, and 16:1ω7c and 18:1ω7c are MUFAs produced by Gr- bacteria under normal (no stress) conditions during metabolism. Please note that a higher ratio from Eq. 1 indicates greater stress. However, the stress indicator for the current study was calculated as:

$$Stress = \frac{(16:1\omega 7c + 18:1\omega 7c)}{(17:0cyclo + 19:0cyclo)} \quad (2)$$

where 17:0cyclo and 19:0cyclo are fatty acids produced by Gr- bacteria under stressful conditions during metabolism, and 16:1ω7c and 18:1ω7c are MUFAs produced by Gr- bacteria under normal (no stress) conditions during metabolism. Please note that a lower ratio from the above equation means greater stress. Eq. (2) was used in this study to more accurately reflect the presence of stress as a function of normal conditions. This is a more intuitive method to compare stressful conditions to normal conditions.

2.6. Statistical analysis

Statistical analysis was conducted by considering the experimental design of the study (split-split plot with a completely randomized whole plot) and the data distribution. To ensure uniform distribution, the Anderson-Darling normality test was selected. Based on the experimental design, Analysis of Variance (ANOVA) was selected to determine treatment, depth, and year effects on measured soil properties. The whole plot factor in this split-split plot experimental design was the vegetative management (CCs vs NC), the split-plot factor was the two years (2021 and 2023) during which this study was conducted, while the split-split plot factor was the soil depths (0–10, 10–20, and 20–30 cm depths). The Anderson-Darling normality test showed that all data were normally distributed ($p = 0.05$). Analysis of variance (ANOVA) was conducted using SAS ver. 9.4 (SAS Institute, 2015) and the PROC GLM procedure for the evaluation of the main effects of CCs and soil depth on measured soil physicochemical properties and microbial biomass, composition, and ratios during both years. For soil depth data analysis, the Tukey's LSD post-hoc analysis was used for comparison. Further, ANOVA was conducted on soil samples collected from the CC plots alone during 2021 and 2023 to analyze any temporal effects of CCs on the measured soil properties, and to determine the year × soil depth interaction effects. Statistical differences were declared at the $\alpha \leq 0.05$ level.

3. Results

3.1. Soil physical and chemical properties

The mean (±SE) and ANOVA results for soil physicochemical properties relative to soil depth as influenced by treatment during 2021 and 2023 is shown in Table 3. During 2021, when averaged over all sampling depths, the soil TN and pH were 11 % and 4 % greater, respectively, under CC management compared with NC management. Besides Θ and Ca, soil sampling depth significantly affected all soil properties analyzed when averaged over all treatments. Soil BD increased with increasing soil depth, whereas all other measured properties decreased with increasing soil depth (Table 3).

Table 2
Phospholipid fatty acid biomarkers used by the University of Missouri Soil Health Assessment Center for microbial group identification.

	Chemical Groups				
Category	**Branched**	**10-methyl**	**Cyclo**	**PUFA**	**MUFA**
Actinomycetes		16:0, 17:1 w7c, 17:0, 18:1 w7c, 18:0,			
Arbuscular mycorrhizal fungi		16:1 w5c			
Bacteria	11:0 iso, 12:0 iso, 12:0 antesio, 13:0, 14:0, 14:1ω9c		17:0c, 18:0c		
Eukaryotes				15:4 w3c, 15:3 w3c, 16:4 w3c, 16:3 w6c, 18:3 w6c, 18:4 w3c,	
Fungi			18:2 w6c,		
Gr+	11:0 iso, 11:0 anteiso, 12:0 iso, 12:0 anteiso, 13:0 iso, 13:0 anteiso, 14:0 iso, 14:0 anteiso, 15:1 anteiso w9c, 15:0 iso, 15:0 anteiso, 16:0 iso, 16:0 anteiso,		19:0 cyclo w9c,	15:1 iso w9c, 15:1 iso w6c,	14:1 iso w7c,
Gr-			17:0 cyclo w7c, 19:0 cyclo w7c, 19:0 cyclo w6c, 20:0 cyclo w6c		12:1 w8c, 12:1 w5c, 13:1 w5c, 13:1 w4c, 13:1 w3c, 14:1 w9c,

Please note: Gr+ = gram positive bacteria, Gr- = gram negative bacteria.

In 2023, when averaged over all sampling depths, the SOC, Θ, TN, P, and Mg values were 54 % (Fig. 2a), 17 %, 118 % (Fig. 2b), 210 %, and 28 % greater, respectively, under CC management than NC management. Conversely, during the same period, the soil depth-averaged BD was 14 % greater under NC management than CC management. Besides K, Ca, and soil pH, the treatment averaged soil properties were significantly affected by soil depth. Soil properties significantly decreased with increasing soil depth. Conversely, BD increased with increasing soil depth (Table 3).

A comparison of the CC treatments alone between 2021 and 2023 showed that SOC, Θ K, and Mg were 24 %, 30 %, 37 %, and 21 % greater, respectively, during 2023 compared to 2021. Contrarily, BD was 8 % greater in 2021 than in 2023 (Table 3, Fig. 3).

3.2. Microbial biomass and community composition

Table 4 shows the mean (±SE) and ANOVA data for soil microbial biomass and community composition by soil depth as influenced by treatment during 2021 and 2023. The depth-averaged population of actinomycetes was 13 % greater under CC compared than under NC management during 2021. Although not statistically significant, all other microbial population and biomass were numerically greater under CC management than under NC management. Also in 2021, the treatment-averaged microbial biomass and microbial community composition decreased with increasing soil depth.

During 2023, the values for depth-averaged microbial biomass, total PLFAs, AMF, and fungi were 22 % (Fig. 2c), 8 % (Fig. 2d), 34 %, and 22 % greater, respectively, under CC management than under NC management. The other measured microbial populations were numerically greater under CC management than under NC management. During this period, all microbial biomasses and microbial community populations decreased with increasing soil depth when averaged over both treatments (Table 4).

The effects of CCs over time were measured by comparing the soil properties under CC management alone between 2021 and 2023. Under CC management, the depth-averaged microbial biomass, total PLFAs, AMF, fungi, and Gr+ were 20 %, 6 %, 39 %, 48 %, and 8 %, greater, respectively, in 2023 than in 2021 2021. Although not statistically significant, Gr-, eukaryotes, and actinomycetes were greater in 2023 than in 2021 (Table 4, Fig. 4).

3.3. Microbial ratios

Table 5 shows the mean (±SE) and ANOVA data for microbial ratios with respect to soil depth for the two treatments during the study period. During 2021, the depth averaged mono:poly and status:stress ratios were 13 % and 38 % greater, respectively, under CC management than under NC management. Although not statistically significant, other measured microbial ratios were greater under CC management than under NC management. During 2021, all treatment-averaged microbial ratios decreased with increasing soil depth.

During 2023, the depth-averaged F:B, Pred:Prey, mono:poly, and status:stress ratios were 22 % (Fig. 2e), 200 % (Fig. 2f), 49 %, and 38 %, greater, respectively, under CC management than under NC management (Table 5). Although not statistically significant, the Gr+ :Gr-, sat:unsat, and MUFA:Branched ratios were greater under CC management than under NC management. During 2023, all treatment-averaged microbial ratios measured decreased with increasing in soil depth.

A comparison of the microbial ratios under CC management between 2021 and 2023 showed that the depth-averaged F:B, Pred:Prey, mono:poly, and MUFA:Branched ratios were 22 %, 50 %, 37 % and 25 %, greater, respectively, during 2023 than during 2021. Conversely, the sat:unsat ratio was 46 % greater during 2021 than during 2023 (Table 5, Fig. 5).

Table 3
Mean (±SE) of measured soil physicaochemical properties with respect to soil depth and treatments.

Treatment	SOC (g kg^{-1})	BD (g cm^{-3})	Θ (cm^3 cm^{-3})	TN (g kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	Mg (mg kg^{-1})	Ca (mg kg^{-1})	pH
2021									
CC	24.23 ± 2.33	1.33 ± 0.03	0.27 ± 0.02	29.53 ± 1.19a	17.79 ± 5.00	204.21 ± 2.82	10.60 ± 0.41	82.73 ± 6.52	6.67 ± 0.03a
NC	23.07 ± 1.79	1.36 ± 0.04	0.25 ± 0.03	26.49 ± 1.14b	15.22 ± 3.52	200.50 ± 0.70	10.06 ± 0.40	80.34 ± 4.56	6.39 ± 0.06b
Depth (cm)									
0–10	27.70 ± 2.55a	1.27 ± 0.04b	0.29 ± 0.03	31.16 ± 0.97a	25.00 ± 3.35a	205.64 ± 1.37a	11.26 ± 0.30a	86.92 ± 6.45	6.63 ± 0.09a
10–20	22.87 ± 2.04b	1.35 ± 0.03ab	0.26 ± 0.03	28.10 ± 1.00b	14.80 ± 4.99b	202.77 ± 0.35ab	10.68 ± 0.30a	79.92 ± 4.75	6.57 ± 0.05a
20–30	18.89 ± 1.75c	1.42 ± 0.03a	0.24 ± 0.03	24.77 ± 1.38c	9.72 ± 5.53b	198.66 ± 3.92b	9.05 ± 0.36b	77.77 ± 8.90	6.38 ± 0.07b
ANOVA p > F									
Treatment	0.138	0.242	0.441	0.018	0.379	0.174	0.115	0.702	0.003
Depth	0.001	0.043	0.421	0.002	0.004	0.041	0.005	0.465	0.007
Tmt x depth	0.771	0.880	0.984	0.511	0.873	0.463	0.834	0.867	0.442
2023									
CC	30.01 ± 1.75a	1.23 ± 0.03b	0.35 ± 0.02a	30.51 ± 1.63a	18.05 ± 5.14a	279.08 ± 0.34	12.83 ± 1.14a	99.95 ± 7.75	6.59 ± 0.03
NCC	19.53 ± 1.75b	1.40 ± 0.02a	0.30 ± 0.01b	13.98 ± 0.65b	5.82 ± 2.19b	275.69 ± 0.11	9.99 ± 0.54b	86.71 ± 9.53	6.43 ± 0.06
Depth (cm)									
0–10	30.16 ± 2.52a	1.22 ± 0.05c	0.35 ± 0.02a	25.10 ± 4.65a	21.50 ± 5.09a	280.06 ± 0.39	13.89 ± 1.24a	102.57 ± 8.16	6.59 ± 0.09
10–20	24.96 ± 2.22b	1.33 ± 0.05b	0.33 ± 0.02ab	22.12 ± 3.50ab	8.42 ± 5.37b	270.02 ± 0.24	10.51 ± 0.87b	94.40 ± 8.14	6.52 ± 0.04
20–30	19.18 ± 2.79c	1.40 ± 0.04a	0.29 ± 0.02b	19.53 ± 3.37b	5.88 ± 3.78b	269.57 ± 0.31	9.83 ± 0.91b	88.12 ± 15.77	6.42 ± 0.05
ANOVA p > F									
Treatment	< 0.001	< 0.001	0.052	< 0.001	0.027	0.186	0.017	0.098	0.058
Depth	< 0.001	< 0.001	0.044	0.007	0.047	0.331	0.016	0.345	0.235
Tmt x depth	0.623	0.217	0.494	0.074	0.763	0.667	0.360	0.675	0.957
2021 vs 2023									
Depth (cm)									
0–10	32.43 ± 2.53a	1.20 ± 0.04b	0.35 ± 0.03a	33.91 ± 1.22a	28.65 ± 4.03	242.28 ± 16.74	14.01 ± 1.21a	88.69 ± 8.64	6.70 ± 0.02a
10–20	26.76 ± 2.07ab	1.27 ± 0.03b	0.30 ± 0.02ab	29.39 ± 0.96b	14.84 ± 6.32	241.24 ± 17.52	11.12 ± 0.73b	85.74 ± 5.96	6.65 ± 0.03ab
20–30	22.16 ± 2.03b	1.37 ± 0.03a	0.28 ± 0.02b	26.76 ± 1.51b	10.27 ± 5.52	237.36 ± 19.48	9.96 ± 0.74b	76.15 ± 11.17	6.54 ± 0.05b
ANOVA p > F									
Year	0.030	0.007	0.007	0.532	0.971	< 0.001	0.023	0.580	0.080
Depths	0.013	0.003	0.097	0.008	0.120	0.335	0.006	0.644	0.025
Year x depth	0.991	0.756	0.862	0.638	0.967	0.279	0.157	0.919	0.864

Please note: CC = cover crops, NC = no cover crop, tmt = treatment.
SOC = soil organic carbon, BD = bulk density, Θ = volumetric water content, TN = total nitrogen, P = phosphorous, K = potassium, Mg = magnesium, Ca = calcium.

4. Discussions

4.1. Soil physical and chemical properties

The SOC forms a pool of organic compounds (plant and animal residues) at different stages of decomposition in the soil, making it a vital soil health indicator that directly determines soil quality, sustainability, and food production. Soil organic carbon also affects soil properties (e.g., soil physicochemical and biological properties) that directly determine the soil structure, and therefore, water and nutrient movement and retention (Kumar et al., 2022). As a dynamic soil component, SOC varies over time, and this temporal variability can be influenced by several factors including land management practices and vegetation type (Wang et al., 2021). The results of the current study confirmed an increase in SOC of 54 %, during 2023, under CC management compared with NC management at all sampled soil depths (Fig. 2a). This change probably reflects 1) the addition of diverse plant residues under CC management (Ghimire et al., 2017), and 2) the lack of plant residue addition under NC management (Duval et al., 2016). The microbial degradation of whatever plant residue was available under NC management, coupled with the lack of residue return, was probably responsible for the significant differences in SOC observed between the treatments in 2023 vs. 2021. Overall, the findings presented here indicate that annual cover cropping can improve SOC stocks and increase the soil health benefits associated with SOC.

The significantly greater SOC values in 2023 compared with 2021 in response to CC management (Fig. 3a) confirmed that the continuous addition of aboveground and belowground biomass can improve SOC stocks during prolonged practice. However, a meta-analysis of 181 observations by McClelland et al. (2021) reported no significant difference in SOC over several years of CC management. The reasons for these contradictory finding could include 1) differences in CC biomass (i.e., a positive relationship between biomass and SOC), 2) the method of CC termination, 3) the soil texture, 4) the climate, and 5) the type of CC management (continuous, overwintering, or summer CCs) (Vukicevich et al., 2016). Specifically, the CC biomass used in this study was greater than those reported by McClelland et al. (2021) and was possibly the reason for the contradictory findings. This therefore means that CC biomass return can significantly determine SOC content in soils, and this represents a method for improving soil health. However, current results and those of McClelland et al. (2021) both reported decreases in SOC values relative to increasing soil depth. This decrease in SOC with increasing soil depth can be explained by the reduction in plant residues (including plant roots) with increasing soil depth.

As an important soil health parameter, SOC can influence other soil properties like soil BD, an important parameter for understanding soil ecosystem functions (Al-Shammary et al., 2018). The presence of SOC has been reported to significantly lower BD values (Blanco-Canqui et al., 2009) due to its lower mass to volume ratio compared to that of soil minerals. Soil organic carbon can also increase soil aggregation (Liu et al., 2021) and porosity (Simonetti et al., 2017), and these increases can further lower BD values under CC than NC management. Therefore,

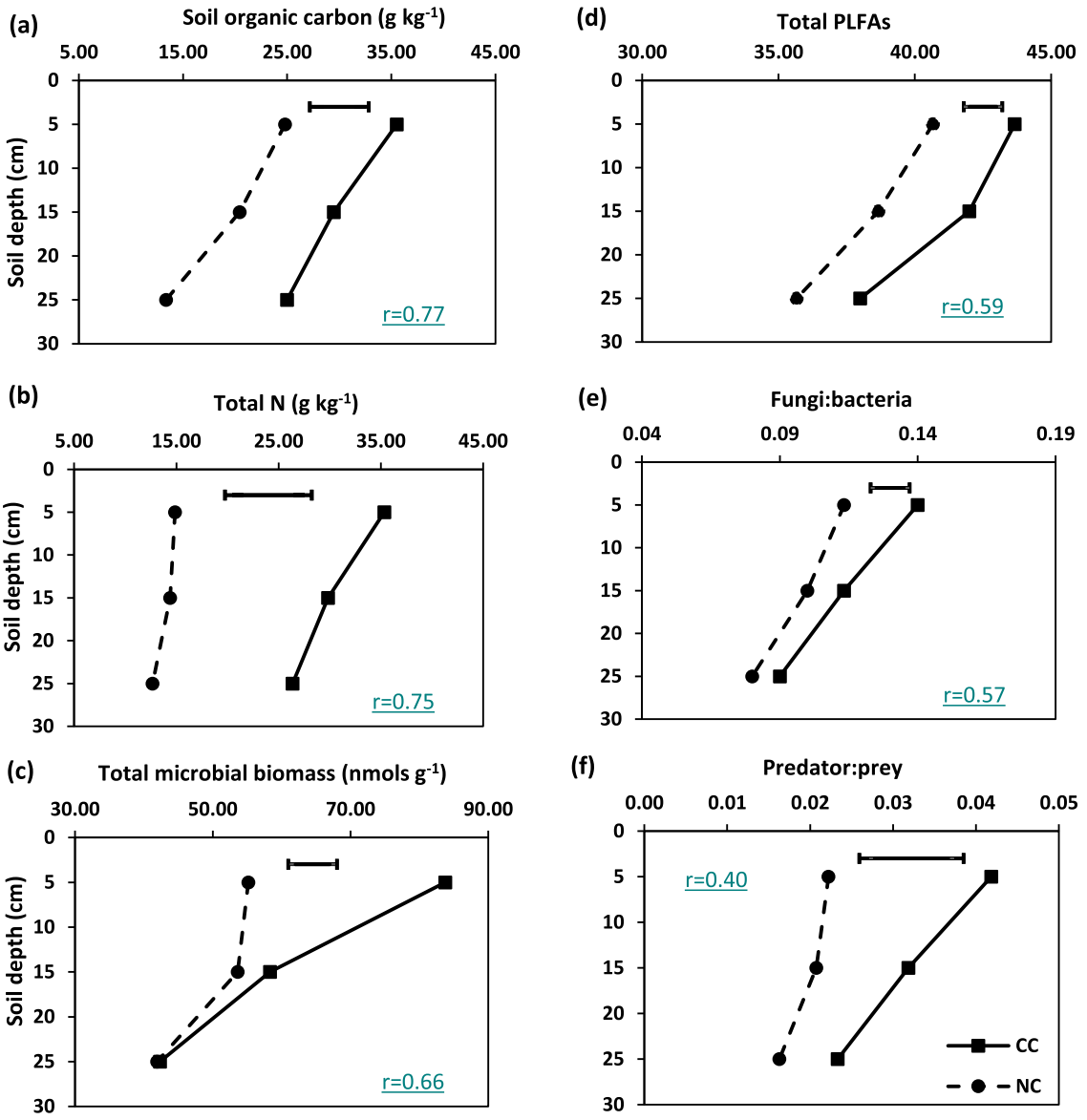

Fig. 2. Management effects on selected soil physicochemical and biological properties relative to soil depth during 2023 alone. Please note that PLFAs = phospholipid fatty acids. Bars represent least squares difference at $p \leq 0.05$. Effect sizes are denoted by Pearson's correlation coefficient (r).

the significantly greater SOC values under CC management was one of the mechanisms responsible for the lower BD values under CC than NC management during 2023 (r = 0.89).

Another mechanism that may have significantly reduced soil BD under CC than NC management is the aboveground and belowground activities of the CCs. The aboveground CC biomass (leaves and terminated residues) can also intercept rain drops (Thiébeau et al., 2021; Haruna et al., 2022), thereby significantly lowering the kinetic energy of the drops before they reach the soil surface. This can reduce soil consolidation, and therefore soil BD, especially in humid environments similar to the study site. The roots of CCs can also increase soil porosity (Abdollahi et al., 2014) thereby lowering soil BD compared with soils under NC management.

An increase in these aforementioned mechanisms over the study period probably gave rise to the significantly lower BD values observed in the CC treatment during 2023 compared with 2021 (Fig. 3b). This means that, over time, CCs could replace conventional tillage to alleviate soil compaction. The decrease in SOC (Table 3), and the weight of overburden soil was probably responsible for the increase in BD with increasing soil depth. Soil BD provides information about the proximity of soil particles to each other, and this property has been utilized by several researchers as a surrogate for soil compaction stress (Stolf et al., 2011; Defossez et al., 2003; Panagos et al., 2024). Overall, CCs can reduce soil compaction stress, and this can reduce the stress on the succeeding cash crop roots, thereby increasing the likelihood of plant root penetration and survival. In addition, the effects of reduced soil compaction would also extend to soil water infiltration, retention, and availability even after CC termination (Haruna et al., 2022).

Water availability is the most limiting factor in plant growth and development and is an important soil physical property necessary for a functional soil ecosystem. One study on the effects of CCs on in situ measured soil water potential by Peters and Haruna (2024a) reported that the biomass provided by CC significantly lowered soil temperature (especially during the 2 months prior to their termination) compared with NC. Consequently, the evaporation of soil water was much lower (and thus soil water content was much higher) under CC management than under NC management. Peters and Haruna (2024a) also reported that water transpiration by CCs reduces antecedent soil water content,

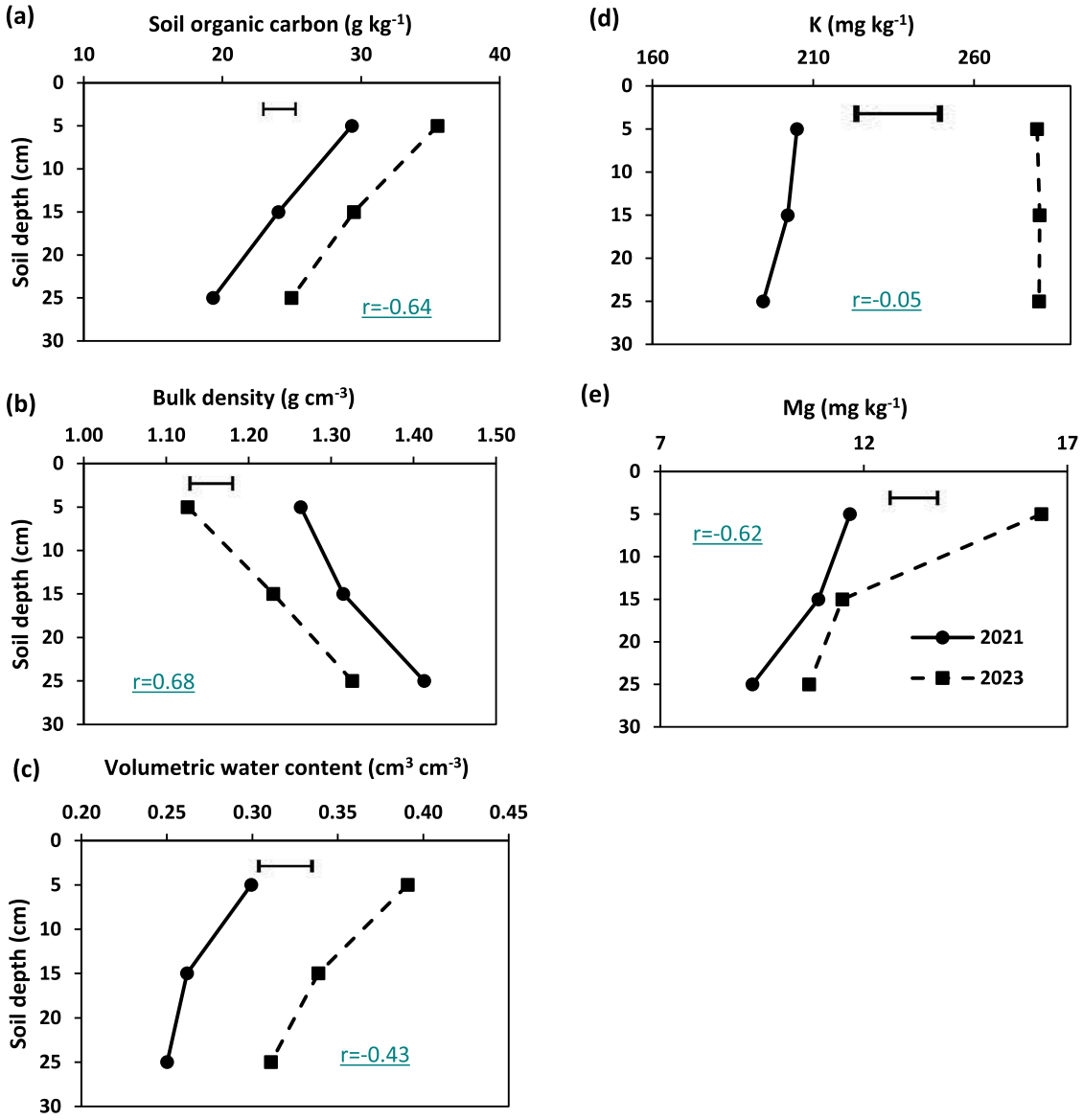

Fig. 3. Soil physcochemical properties under CC management alone during 2021 and 2023 as a function of soil depth. Please note that K = potassium, and Mg = magnesium. Bars represent least squares difference at $p \leq 0.05$. Effect sizes are denoted by Pearson's correlation coefficient (r).

and this often leads to better water infiltration after rainfall or irrigation events. The results from the current study showed that a combination of lower BD and probably less water evaporation led to greater Θ under CC management than under NC management. Furthermore, water transpiration by CCs (leading to lower antecedent soil water content) and subsequent rainfall could also have contributed to the greater water content in the CC treatment than in the NC treatment.

The greater Θ under CC management could also have arisen due to the colloidal properties of SOC, as indicated by the positive relationship between Θ and SOC (Lal, 2020). This finding was similar to the results of Gabriel et al. (2021). Conversely, Vujić et al. (2021) reported that winter CCs significantly reduced soil water content and storage during the spring season. The discrepancy in these results can be attributed to interactions between climate, soil type, and CC biomass. Therefore, careful management like proper CC species selection and termination timing, is encouraged if CCs are to be used to improve water content.

The increases in water content observed over time in the CC treatment suggest that the benefits of SOC accumulation, lower BD, and annual CC roots can improve the water content in soils by increasing proximity between soil particles. This disproved the first hypothesis probably because soil water content and availability is also dependent on soil characteristics as well as plant processes (like transpiration). Therefore, the benefits of CCs in terms of improving soil water content may increase over time in climatic conditions like those in the present study. Since soil Θ determines nutrient movement and solubility (Xue et al., 2017), CC effects on Θ can also extend to nutrient availability.

Nutrient availability is an important determinant of crop productivity and environmental sustainability. One of the benefits of CCs is their reported ability to scavenge and recycle excess nutrients, particularly during periods when the rate of precipitation exceeds the rate of evapotranspiration (Hirsh et al., 2021). This recycling is especially important because not all applied nutrients are utilized by the cash crops that precede the CCs. For example, Hirsh and Weil (2019) reported that an average of 253 kg ha^{-1} of mineral N remained at the end of the growing season in the upper 200 cm of soil in the Mid-Atlantic region of the U.S. However, the extent to which soil nutrients are scavenged will depend on several factors, including the types of CCs used, the time of establishment (and if the CCs are well established), the amount of

Table 4
Mean (±SE) of measured soil microbial biomass community composition with respect to soil depth and treatments.

Treatment	Total microbial biomass (nmol g^{-1})	Total PLFAs	AMF (nmol g^{-1})	Gr- (nmol g^{-1})	Eukaryotes (nmol g^{-1})	Fungi (nmol g^{-1})	Gr+ (nmol g^{-1})	Actinomycetes (nmol g^{-1})
2021								
CC	51.03 ± 3.38	38.78 ± 0.90	1.92 ± 0.30	16.68 ± 2.03	0.62 ± 0.08	0.56 ± 0.13	11.47 ± 0.50	7.89 ± 0.64a
NCC	48.72 ± 2.88	37.44 ± 1.32	1.72 ± 0.25	15.61 ± 2.21	0.51 ± 0.06	0.48 ± 0.08	10.23 ± 1.22	7.00 ± 0.35b
Depth (cm)								
0–10	58.27 ± 2.53a	40.50 ± 1.23a	2.74 ± 0.15a	23.28 ± 1.72a	0.72 ± 0.09a	0.88 ± 0.09a	13.32 ± 1.21a	8.68 ± 0.48a
10–20	51.03 ± 2.19a	38.17 ± 1.28ab	1.63 ± 0.23b	14.74 ± 1.10b	0.56 ± 0.08ab	0.42 ± 0.05b	10.72 ± 0.67b	7.37 ± 0.59b
20–30	40.33 ± 2.05b	35.67 ± 0.95b	1.09 ± 0.09b	10.42 ± 0.67b	0.41 ± 0.04b	0.25 ± 0.05b	8.52 ± 0.41b	6.30 ± 0.50c
ANOVA p > F								
Treatment	0.448	0.231	0.398	0.520	0.165	0.302	0.179	0.014
Depth	0.002	0.012	0.004	0.002	0.020	< 0.001	0.004	0.003
Tmt x depth	0.751	0.967	0.927	0.733	0.671	0.100	0.325	0.165
2023								
CC	61.47 ± 6.66a	41.22 ± 1.06a	2.66 ± 0.34a	20.06 ± 2.55	0.71 ± 0.10	0.83 ± 0.11a	12.39 ± 1.01	8.58 ± 0.93
NCC	50.23 ± 2.44b	38.33 ± 1.00b	1.98 ± 0.27b	17.42 ± 1.71	0.56 ± 0.03	0.68 ± 0.07b	11.37 ± 1.05	7.34 ± 0.44
Depth (cm)								
0–10	69.47 ± 7.16a	42.17 ± 1.01a	3.26 ± 0.29a	24.73 ± 2.82a	0.79 ± 0.10a	1.05 ± 0.06a	14.72 ± 1.01a	9.84 ± 0.77a
10–20	55.95 ± 3.02b	40.33 ± 0.99a	2.30 ± 0.27b	18.03 ± 1.42b	0.67 ± 0.08a	0.75 ± 0.06b	11.77 ± 0.85b	7.91 ± 0.51b
20–30	42.13 ± 2.10c	36.83 ± 1.19b	1.40 ± 0.14c	13.45 ± 0.90b	0.45 ± 0.03b	0.47 ± 0.07c	9.16 ± 0.67b	6.13 ± 0.74b
ANOVA p > F								
Treatment	0.012	0.007	0.014	0.286	0.065	0.028	0.363	0.088
Depth	0.004	0.001	0.002	0.009	0.011	< 0.001	0.006	0.003
Tmt x depth	0.022	0.886	0.436	0.849	0.285	0.312	0.959	0.401
2021 vs 2023								
Depth (cm)								
0–10	72.38 ± 6.32a	41.83 ± 1.01a	3.32 ± 0.28a	24.39 ± 3.06a	0.85 ± 0.12a	1.10 ± 0.03a	13.47 ± 1.00a	9.85 ± 0.86a
10–20	54.75 ± 3.33b	39.67 ± 1.41a	2.21 ± 0.30b	16.85 ± 1.66b	0.72 ± 0.09a	0.63 ± 0.11b	11.14 ± 0.78b	7.68 ± 0.68ab
20–30	41.62 ± 1.91c	36.50 ± 0.96b	1.35 ± 0.16c	12.26 ± 1.17b	0.43 ± 0.03b	0.35 ± 0.09c	9.31 ± 0.65b	5.85 ± 0.78b
ANOVA p > F								
Year	0.018	0.002	0.007	0.089	0.428	0.001	0.023	0.096
Depths	< 0.001	0.003	< 0.001	0.005	0.022	< 0.001	0.005	0.010
Year x depth	0.100	0.785	0.630	0.954	0.894	0.123	0.610	0.726

Please note: CC = cover crops, NC = no cover crop, tmt = treatment.
PLFA = phospholipid fatty acid, AMF = arbuscular mycorrhizal fungi, Gr- = gram negative bacteria, Gr+ = gram positive bacteria.

biomass generated, and the residue management methods.

During the study period, the multi-species CCs used generated a substantial amount of biomass. Both legume and non-legume CCs can serve as catch crops, especially for very mobile nutrient elements like N (Zuk-Golaszewska et al., 2019; Vogeler et al., 2019). Cover crops have also been demonstrated to acquire P through several strategies including the mobilization of meagerly soluble P (organic and inorganic forms) and the mineralization of organic P (Hallama et al., 2019). These scavenged nutrients can be released into the soil upon their incorporation (Abdalla et al., 2019). Therefore, the significantly greater TN (Fig. 2b), P ($r = 0.22$), and Mg ($r = 0.23$) under CC management than under NC management during 2023 can be attributed to the recycling of these nutrients by CCs on the one hand, and the lack of CC biomass under NC management on the other hand. The cumulative effects of these opposing mechanisms lead to more nutrient accumulation under CC management and less accumulation under NC management. The strong effect of CC management on TN probably has more to do with nutrient cycling than atmospheric N fixation since over 60 % of the CCs utilized in the present study were non-legumes. Although the differences in K, and Ca between CC and NC management were not statistically significant, the contents were greater under CC management than under NC management. Since K and Ca are base forming cations, this property may have contributed to the significantly greater soil pH under CC management than under NC management during 2021 and 2023 (Table 3).

Another notable finding was that TN was the only measured soil nutrient that showed significantly greater levels under CC management than under NC management during 2021. This did not support the second hypothesis because about 38 % of the CCs used were legumes, suggesting that 1) the symbiotic addition of N outweighs nutrient scavenging, 2) the onset of symbiotic N addition occurs earlier than scavenging (since the fields were established in 2020 and 2021 was essentially the first year of the study), or 3) both processes complement each other. Regardless, the results of the current study suggests that the use of multi-species CCs may benefit soil nutrient management and availability.

Under CC management, the soil K and Mg contents were significantly greater during 2023 than during 2021, especially at the 0–10 cm soil depth (Fig. 3d&e). This suggests a significant recycling of these nutrients by the CCs since the rate of nutrient application did not change during the study period.

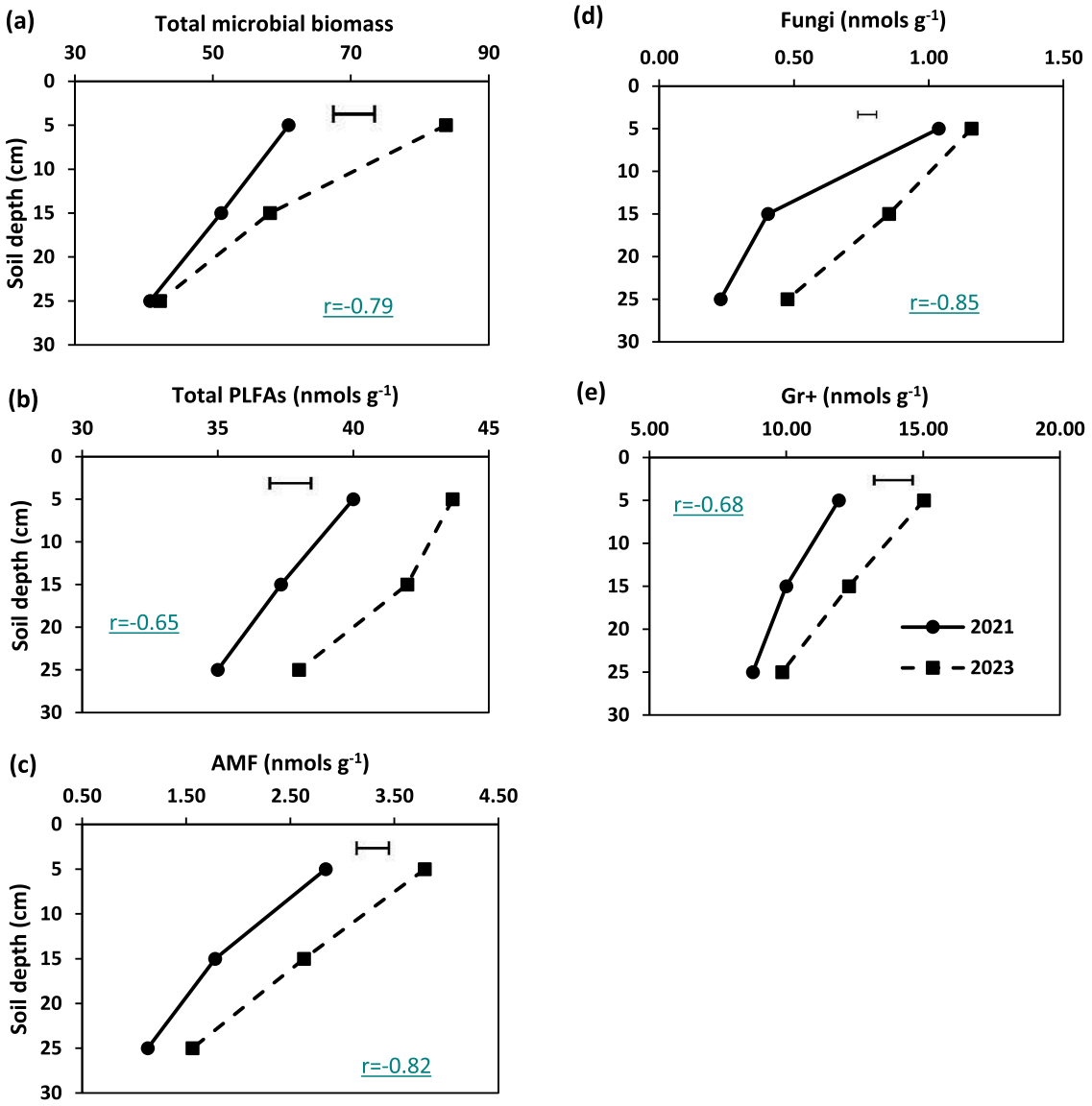

Fig. 4. Soil total microbial biomass and microbial biomass composition under CC management alone during 2021 and 2023 as a function of soil depth. Please note that PLFA = phospholipid fatty acids, AMF = arbuscular mycorrhizal fungi, and Gr+ = gram positive bacteria. Bars represent least squares difference at $p \leq 0.05$. Effect sizes are denoted by Pearson's correlation coefficient (r).

4.2. Microbial biomass and community composition

The soil microbial biomass represents the living component of soil organic matter (less than 5 % of soil organic matter), and soil microorganisms play a vital role in soil nutrient availability and cycling (e.g., N, C, S, and P), and soil physical stability (Sithole et al., 2016). Nevertheless, microbial biomass results are neither perfect nor absolute, but instead serve as a vital indicator of soil function in regard to agroecosystem use and management. As such, the soil microbial biomass and community composition are often used as indicators of soil management effects on the physicochemical properties of soils (Gayan et al., 2023).

Soil physicochemical properties, such as aggregate stabilization, and N and P solubility/availability, are determined by actinomycetes, which are aerobic and spore-forming bacteria that thrive under near neutral soil pH conditions (Bhatti et al., 2017). The significantly greater actinomycetes population under CC management than under NC management during 2021 was attributed to the significantly higher soil pH under CC management. Consequently, the actinomycetes may have contributed, through symbiotic and non-symbiotic means, to the greater availability of N under CC management than under NC management. Furthermore, their filamentous nature and ability to stabilize soil aggregates could allow actinomycetes to play an important role in soil health improvement. Therefore, by promoting the actinomycete population in soils, CCs can improve soil health, and therefore soil microbial biomass.

The significantly greater soil microbial biomass (Fig. 2c), total PLFA contents (Fig. 2d), and microbial composition (AMF and fungi) under CC management than under NC management during 2023 can be attributed to the improved soil health parameters (improved SOC, Θ, and lower BD) achieved under CC management. Specifically, as obligate symbionts, AMF is important for water and nutrient (particularly P) transfer between the soil and plants (Dsouza, 2019). This nutritional symbiosis occurs as an exchange of P for C and can lead to improvements in SOC. As a result, this symbiosis is another possible mechanism that could explain the improved SOC values observed under CC management compared with NC management. This also seems to suggest that CCs can improve nutrient transfer (especially P) in the subsequent commodity crops due to the CC effects on AMF populations. Exudates from the CC

Table 5
Mean (±SE) of measured soil microbial ratios composition with respect to soil depth and treatments.

Treatment	F:B	Pred:Prey	Gr+ :Gr-	Sat:Unsat	Mono:Poly	Status:Stress	MUFA:Branched
2021							
CC	0.09 ± 0.01	0.02 ± 0.01	0.54 ± 0.05	2.12 ± 0.21	9.20 ± 0.80a	1.16 ± 0.13a	0.81 ± 0.07
NCC	0.08 ± 0.01	0.01 ± 0.01	0.53 ± 0.04	1.81 ± 0.16	8.12 ± 0.51b	0.86 ± 0.04b	0.70 ± 0.04
Depth (cm)							
0–10	0.10 ± 0.01a	0.02 ± 0.01	0.59 ± 0.04a	2.33 ± 0.14a	9.69 ± 0.80a	1.25 ± 0.17a	0.91 ± 0.08a
10–20	0.08 ± 0.01b	0.02 ± 0.01	0.56 ± 0.05a	1.95 ± 0.17ab	8.34 ± 0.75b	0.98 ± 0.08b	0.73 ± 0.03b
20–30	0.07 ± 0.01b	0.02 ± 0.01	0.46 ± 0.07b	1.63 ± 0.28b	7.94 ± 0.89b	0.80 ± 0.03b	0.63 ± 0.04b
ANOVA p > F							
Treatment	0.415	0.454	0.713	0.152	0.039	0.007	0.058
Depth	0.006	0.212	0.003	0.045	0.026	0.006	0.007
Tmt x depth	0.892	0.564	0.327	0.554	0.767	0.060	0.117
2023							
CC	0.11 ± 0.01a	0.03 ± 0.01a	0.64 ± 0.04	1.45 ± 0.13	12.59 ± 0.87a	1.49 ± 0.16a	1.01 ± 0.07
NCC	0.09 ± 0.01b	0.01 ± 0.01b	0.53 ± 0.05	1.33 ± 0.08	8.45 ± 0.62b	1.08 ± 0.04b	0.90 ± 0.06
Depth (cm)							
0–10	0.13 ± 0.01a	0.03 ± 0.01a	0.66 ± 0.05	1.63 ± 0.12a	11.89 ± 1.23a	1.51 ± 0.22a	1.13 ± 0.08a
10–20	0.11 ± 0.01b	0.02 ± 0.01ab	0.58 ± 0.05	1.43 ± 0.10ab	10.24 ± 1.19ab	1.29 ± 0.14ab	0.95 ± 0.04b
20–30	0.09 ± 0.01c	0.01 ± 0.01b	0.52 ± 0.07	1.12 ± 0.07b	9.42 ± 1.33b	1.05 ± 0.05b	0.79 ± 0.03b
ANOVA p > F							
Treatment	0.040	0.003	0.076	0.305	0.001	0.013	0.108
Depth	0.003	0.022	0.154	0.013	0.037	0.046	0.005
Tmt x depth	0.611	0.857	0.702	0.999	0.810	0.296	0.883
2021 vs 2023							
Depth (cm)							
0–10	0.12 ± 0.01a	0.03 ± 0.01a	0.68 ± 0.04a	2.14 ± 0.19a	11.86 ± 1.30	1.72 ± 0.18a	1.11 ± 0.08a
10–20	0.09 ± 0.01b	0.02 ± 0.01ab	0.60 ± 0.04ab	1.82 ± 0.22ab	10.69 ± 1.17	1.28 ± 0.15b	0.89 ± 0.07b
20–30	0.08 ± 0.01b	0.02 ± 0.01b	0.49 ± 0.06b	1.41 ± 0.26b	10.13 ± 1.30	0.98 ± 0.08b	0.73 ± 0.05b
ANOVA p > F							
Year	0.001	0.015	0.101	0.008	0.022	0.062	0.007
Depths	0.001	0.044	0.042	0.044	0.552	0.008	0.001
Year x depth	0.447	0.619	0.916	0.673	0.955	0.985	0.656

Please note: CC = cover crops, NC = no cover crop, tmt = treatment.
F:B = fungi:bacteria ratio, Pred:Prey = predator:prey ratio, Gr+ :Gr- = gram positive bacteria:gram negative bacteria ratio, sat:unsat = saturated:unsaturated phospholipid fatty acids ratio, mono:poly = monounsaturated:polyunsaturated phospholipid fatty acids ratio, status:stress = gram-metabolic status:stress ratio, MUFA:Branched = monounsaturated fatty acids:branched phospholipid fatty acids ratio.

roots can also increase microbial biomass, either directly by acting as a source of energy, or indirectly by serving as catalyst for the breakdown of organic matter (Duan et al., 2022). This possibility could also explain the greater microbial biomass under CC management than under NC management.

Since PLFA analysis is used to quantify total microbial biomass, identify different microbial groups, and assess microbial community structure, it follows logically that, by increasing total microbial biomass, CC management will significantly improve total PLFAs than NC. This is especially important, and more likely, since PLFA analysis is more accurate at differentiating differences in biological markers compared to PCR and CLIP-based methods (Ramsey et al., 2006). The significantly greater total content of PLFAs in the CC treatment than in the NC treatment during 2023 probably reflected the enhanced inputs of aboveground and belowground residues. This possibility is supported by a meta-analysis of 81 studies, where Muhammad et al., (2021) reported that CCs enhanced soil PLFAs by 24 % compared with NC management due to C and N inputs from the CC residues. This demonstrates the activity of living microorganisms because, after microbial cell death, their cell membranes are rapidly degraded and metabolized (Dunfield, 2008). Therefore, total PLFAs can serve as an important indicator of the living microorganism biomass in soil (Zhang et al., 2019). These living microorganisms are important for several ecosystem services such as bioremediation (Singh et al., 2019). Consequently, in addition to the already mentioned soil health benefits of CCs compared with NC management, CCs can also improve soil ecosystem services by increasing the amount of living microorganisms in the soil.

As demonstrated above, CCs can improve total microbial biomass. However, the proportion of various soil microorganism is subject to the availability and type of plant biomass available. In the present study, over 60 % of the CCs used were non-legume species. Researchers have reported that fungi thrive better in C-rich environments with non-legume CCs, whereas bacteria thrive better under legume CCs (Nakamoto et al., 2012; Frasier et al., 2016; Brennan and Acosta-Martinez, 2017). Therefore, the significantly greater fungi population in the CC treatment than under the NC treatment in 2023 was attributed to the incorporation of the biomass greater in the proportion of the non-legume CC species into the soil after desiccation. Soil fungi play an important role in the breakdown and decomposition of lignin and other complex components of organic matter, and this process makes simple sugars available to other microorganisms. The present study findings show that after about three years of CC treatment, nutrient cycling in the soil can be improved due to enhancement of soil fungal populations. Consequently, this can further improve microbial community biomass and diversity through the availability of simple sugars.

Besides the positive influence of CCs on total microbial biomass during a single year (2023), a temporal effect of CCs was also evident on microbial community biomass and composition. Over time, CCs significantly improved SOC and Θ (Fig. 3a&c), and significantly lowered soil BD (Fig. 3b). Seitz et al. (2023) reported that superclass lipids and other lipid-like compounds are exuded from the roots of CCs. In the present study, the CC biomass was greater during 2023 than during 2021, and could possibly have resulted in greater amounts of root exudates during 2023. These benefits may also have contributed to significant improvements in the total microbial biomass, total PLFAs, AMF, fungi, and G+ at various depths (Fig. 4a-e).

Microbial biomass and community composition results did not support the third hypothesis for the current study, probably due to better-than expected CC effects on the physical and chemical soil health indicators. Improvements in Θ and nutrients under CC management than NC may have reduced the expected competition among soil organisms.

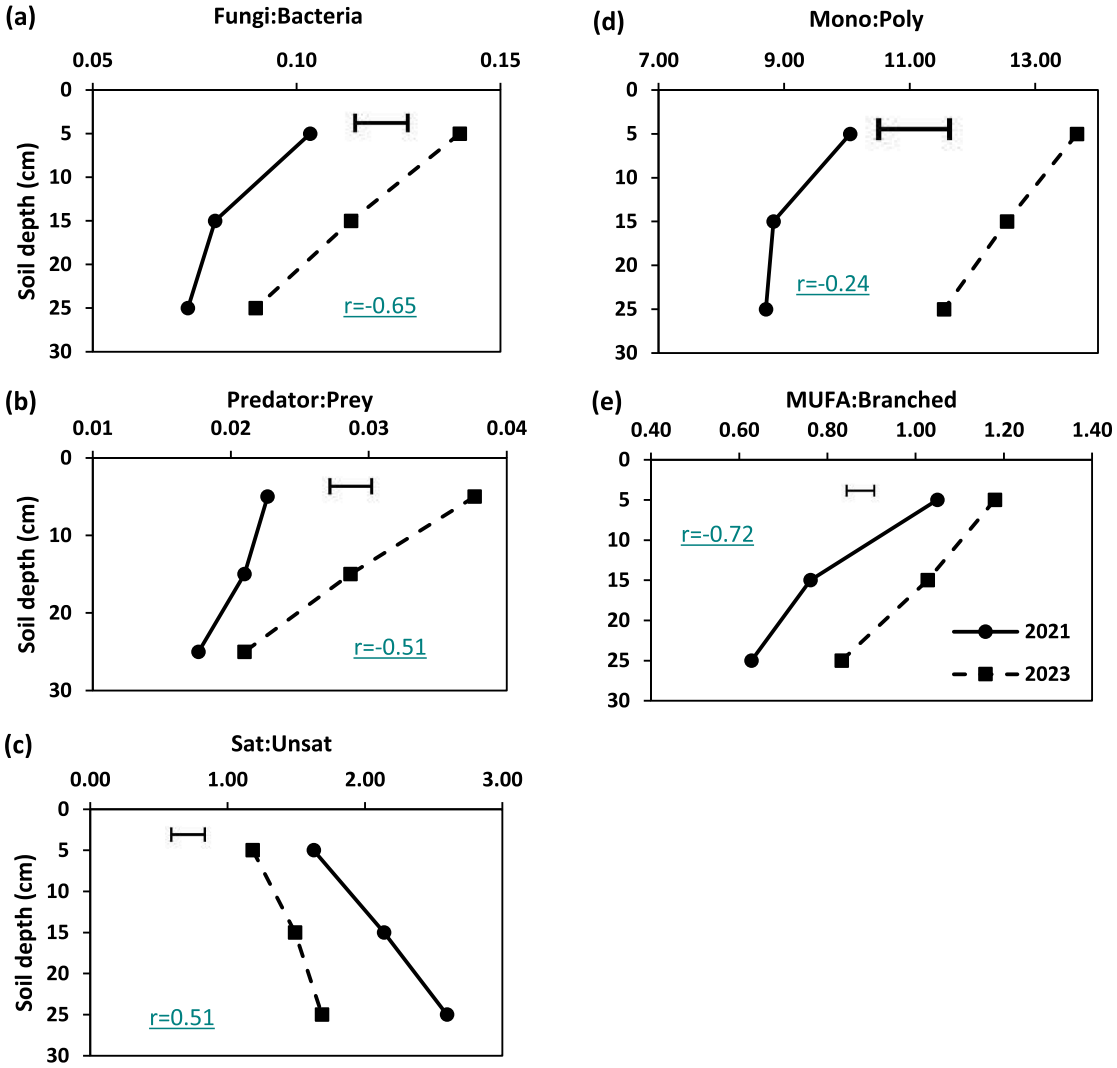

Fig. 5. Microbial ratios under CC management alone during 2021 and 2023 as a function of soil depth. Please note that sat:unsat = saturated:unsaturated phospholipid fatty acid ratio, mono:poly = monounsaturated:polyunsaturated phospholipid fatty acids ratio, and MUFA:Branched = monounsaturated fatty acids: branched phospholipid fatty acids ratio. Bars represent least squares difference at $p \leq 0.05$. Effect sizes are denoted by Pearson's correlation coefficient (r).

In general, this study suggests that nutrient cycling under CC management is a function of the CCs' ability to improve: 1) SOC, and 2) soil microbial biomass and diversity. The SOC improvement arises from the ability of CCs to either scavenge excess nutrients or symbiotically add certain nutrients, and then return their biomass to the soil. Once added, the SOC serves as an energy source that supports increased microbial activity and diversity, and this ultimately leads to the release of soil nutrients. Notably, bacterial populations were similar in both management practices. Since SOC was significantly greater under CC management than under NC management, and the proportion of legume CCs was lower than the proportion of non-legume CCs, this suggests that bacterial biomasses and population are more dependent on the CC type and species than on the total CC biomass.

4.3. Microbial ratios

Microbial ratios are important determinants of many soil factors, including nutrient cycling and decomposition, the presence or absence of soil disturbance, microbial community health, and the presence or absence of stressors in the environment (Kaur et al., 2005; Mills et al., 2020). Therefore, the microbial biomass ratios in the current study were used purely as indices to explain microbial soil health parameters and were not intended to reflect absolute biomass values.

Since fungi are important for the breakdown and decomposition of complex components in organic matter, the greater F:B ratio under CC management further demonstrated that the CC management practice can improve nutrient cycling over that possible with NC management after 3 years (Fig. 2e). A study by de Vries et al. (2006) reported that the F:B ratio can be an index of self-regulation within an ecosystem. Therefore, this study shows that the inclusion of CCs into crop rotation cycles can increase the resilience and sustainability of agroecosystems. This is supported by the SOC, BD, Θ, and microbial community biomass results discussed in the foregoing. Further, Bailey et al. (2002) showed that F:B ratio can be an indicator of C sequestration, with greater F:B indicating more C sequestration. This agrees with the present study findings, where CC management significantly improved SOC and soil nutrients (after 3 years) compared with NC management. This shows that CCs can increase soil function and ecosystem resilience in a changing global climate.

While microorganisms play a vital role in improving soil function, their activity is partly dependent on the proportion of predators to prey. Eukaryotes were classified as predators in this study because they feed on each other, plant residues, and bacteria (Husnik and McCutcheon, 2018). Therefore, both Gr+ and Gr- bacteria were classified as prey.

Eukaryotes are involved in the decomposition of organic matter, soil structure development, and water regulation (Köninger et al., 2023), all of which are important soil health parameters. The Pred:Prey ratio demonstrated that CCs could improve soil health parameters, reduce soil-borne pathogens, and lead to a more diverse and healthier microbial community when compared with NC management at the top 20 cm soil depth (Fig. 2f).

The health of soil microbial community can be limited by the presence or absence of stressors. An important indicator of bacterial stress is the microbial chemical structure characterizations (e.g., mono:poly, and Gr- metabolic status:stress ratios). Francisco et al. (2016) demonstrated that greater sat:unsat, and lower mono:poly and Gr- metabolic status: stress ratios are bacterial stress indicators in agroecosystems. Therefore, the microbial chemical structure characterization results in this study demonstrates that NC management has the potential to increase bacterial stress compared with CC management. Due to the importance of bacteria in N fixation, the argument can be made that NC management can limit the N-fixing efficiency of subsequent commodity crops due to higher bacterial stress.

In the absence of stressors, MUFAs are generally produced by AMF, fungi, and Gr- bacteria, whereas micro eukaryotes produce branched PLFAs. As such, greater MUFAs can be used as an indicator of microbial diversity and health. A comparison of CC management over time during the study period showed that this management practice can improve microbial diversity and health and at different soil depths (Fig. 5a-e) than NC management. Zhang et al. (2018) also showed that MUFA: branched ratio is representative of the proportion of aerobic to anaerobic microorganisms. Therefore, CC management can shift microbial population, over time, toward anaerobic microorganisms by improving soil water content ($r = 0.93$). This possibility is supported by the actinomycete population results from the current study. Under anaerobic conditions, Ueki et al. (2018) reported that anaerobic bacteria (e.g. actinomycetes) can release anti-pathogenic enzymes into the soil which can severely degrade the mycelia of *fusarium*, a pathogen that causes wilts in spinach (*Spinacia oleracea*) and other commodity crops. This can provide a short-term benefit for subsequent commodity crops. However, long-term effects of a shift towards more anaerobic microbial populations may include denitrification, especially in environments and climatic conditions similar to those of the present study. This can be mitigated by effective nutrient application and management strategies.

As expected, the microbial community biomass composition and ratio decreased with increasing soil depth during this study, regardless of the soil management treatment. This was attributed to better soil conditions (SOC and nutrient availability) at the soil surface compared to lower depths.

Contrary to the second and third hypotheses, the current study showed that CC management can improve nutrient availability and cycling, enhance organic matter decomposition, and promote soil microbial diversity. Interestingly, these benefits increased over time, leading to improved soil health and ecosystems services in soils treated with CCs than with NC management in temperate sub humid climates.

While this study was designed to evaluate the benefits of multi-species CCs on soil physical, chemical and biological properties, findings are limited to sub humid climates. It also does not compare benefits of multi-species CCs to single species CC. Admittedly, there are meta-analysis of several studies evaluating this contrast (e.g. Florence and McGuire, 2020; Wojciechowski et al., 2023). However, these studies compare management practices over climatic conditions and soil types and may not be beneficial to producers searching for climate and soil type-specific data. Therefore, future studies should consider evaluating the impact of single vs multi-species CCs on soil health parameters.

5. Conclusions

This study evaluated the effects of CCs on abiotic and biotic soil health indicators. After the three-year study, significantly greater increases in SOC and Θ, and significantly lower soil BD were observed under CC management than under NC management. Although nutrient application during the study period was similar between both management practices, the soil TN, P, and Mg were significantly greater under CC management suggesting the occurrence of nutrient cycling and symbiotic fixation. Nutrient cycling was supported by greater AMF and fungal populations under CC management than under NC management, whereas symbiotic N fixation was supported by the detection of lower mono:poly and Gr- metabolic status:stress ratios in the CC treatment than in the NC treatment. Cover crops also improved soil resilience by increasing the health and diversity of soil microbial population. Also, results showed that the benefits of CCs improved and persisted over the three-years of the present study.

CRediT authorship contribution statement

Md. Ariful Haque: Writing – review & editing, Software. **Seockmo Ku:** Writing – review & editing, Funding acquisition. **Haruna Samuel:** Writing – original draft, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Doofan Eke:** Writing – original draft.

Funding

This study was supported by the Southern Region Sustainable Agriculture Research and Education grant (LS24–391), the TAMU AgriLife Research VFIC program (VFIC23–1001), the USDA—NIFA Capacity Building Grants for Non-Land Grant Colleges of Agriculture program (Grant No. 2023–70001–40998), the Hatch program of USDA NIFA (Grant No. 2021–51106–35583), and the Food Diversity Innovation Program offered by IFANCA.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors are grateful to Mr. Will Hutchinson and the personnel of the Hutchinson farms for permission to conduct this study on their property. Appreciation is also extended to Isabelle Legrain, Jaylan Baker, Imran Khan, Beatrice Neville, and Gray Fogo for the help rendered with soil preparation and analysis.

Data availability

Data will be made available on request.

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