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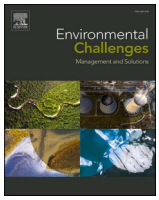

Effects of cover crop on selected abiotic and biotic soil health indicators

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ABSTRACT

Current cropping systems, like cover cropping, aim to improve soil health and crop productivity, with the former a more sustainable route to the latter. This can be done by evaluating the influence of cover crops (CCs) on soil health indicators, both abiotic and biotic, as is the objective of this study. Several CCs (crimson clover [*Trifolium incarnatum* L.], oats [*Avena sativa*], hairy vetch [*Vicia villosa* Roth.], winter wheat [*Triticum aestivum* L.], winter peas [*Lathyrus hirsutus* L.], flax [*Linum usitassimum* L.], triticale [*Triticale hexaploide* Lart.], cereal rye [Secale cereale], and barley [*Hordeum vulgare* L.]) were used across two research sites, set up using a completely randomized design with two levels of CCs (CC vs no cover crop [NC]) and three replicates during 2023. Soil samples were collected at 0–10, 10–20, and 20–30 cm depths and analyzed for soil physico-chemical properties and microbial biomass and composition. Results showed significantly lower bulk density values, greater water content (at 0 kPa soil water matric potential), greater volume-specific heat capacity (at 0 and -33 kPa soil water matric potential), greater total nitrogen, and numerically greater soil organic carbon under CC compared with NC management. This led to numerically greater microbial biomass and community composition (e.g., arbuscular mycorrhizal fungi, gram-negative and gram-positive bacteria, eukaryotes, and fungi), and slightly lower microbial stress indicators (genotypic and chemical structure categorizations) under CC compared with NC management. However, the lack of significant differences between treatments suggests that three years is insufficient to detect improvements in measured soil health indicators. Further, the significant differences in measured soil health indicators between study sites suggests an influence of soil texture and order, and this warrants further investigations.

1. Introduction

Historically, agricultural management practices have primarily focused on improving crop productivity, often neglecting soil quality. However, contemporary efforts on improving agricultural productivity per capita is focused on improving soil health indicators (Haruna and Anderson, 2024). These indicators can be physical, chemical, and biological, with an overwhelming influence and interaction between these indicators and agricultural management practices (Iqbal et al., 2014; Pervaiz et al., 2020; Liptzin et al., 2022; Xu et al., 2022).

The abiotic indicators like soil bulk density (BD), water movement and retention, soil pH, total N, and extractable P and K are influenced by agricultural management practice like cover crops (CCs). For example CCs (equally used as soil primers in some regions and as commodity crops in others) have been reported to significantly lower BD by 3 % (Adeli et al. 2020), 12 % (Haruna, 2019), and as much as 24 % (Nascente et al., 2015) at the 0–10 cm depths compared with no cover crop (NC) management. These benefits were related to CC root activity and soil organic C (SOC) contents. Conversely, Garcia et al. (2013), Teixeira et al. (2016), and Reichert et al. (2019) all reported that CCs had no noticeable difference in BD values at similar depths. Additionally, while Hubbard et al. (2013) found an 18 % increase in water retention at −33 kPa soil water pressures at the top 10 cm soil depth under CC management compared with NC management, Rorick and Kladivko (2017) reported no significant differences in water retention at similar soil water potentials and depth between the two management practices.

Recently, researchers have been examining the effects of CCs on heat transport parameters. For example, Haruna and Anderson (2022) found that CCs reduced thermal conductivity (λ) and increased volume-specific heat capacity (C_V) at the 0–10 cm depths compared with NC management. These authors attributed these changes to the greater BD values under NC management, which led to closer proximity between soil particles for heat transport, and the greater water content and SOC under CC management which enhanced heat buffering capacity. In

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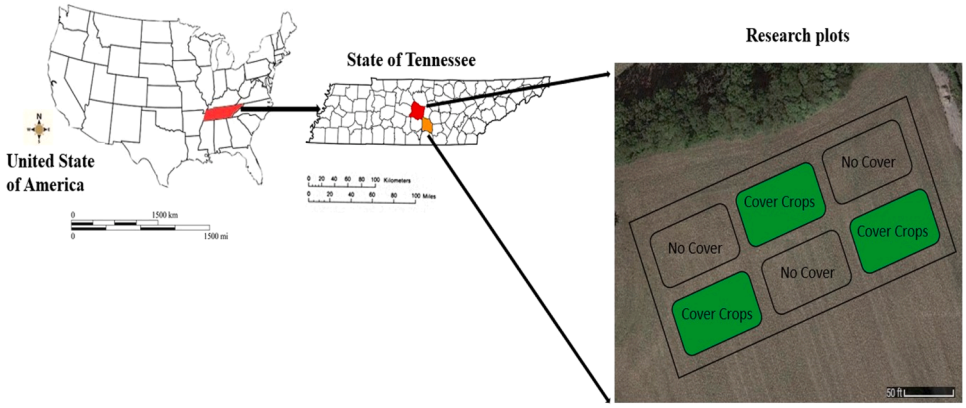

Fig. 1. Research sites in Tennessee showing the research plots. Please note that the red shade on the map of Tennessee depicts the location of the Murfreesboro site, while the orange shade shows the location of the Estill Springs site.

contrast, Sindelar et al. (2019) reported no significant effect of CCs on λ and C_V at similar soil depths, attributing this to the absence of significant effect of CCs on soil BD, SOC and water content.

Several researchers have identified a linear relationship between soil pH, SOC, N, K, and P (Abdollahi and Munkolm, 2014; Ensinas et al., 2016; Khan et al., 2021). However, many studies were unable to detect any significant effect of CCs on soil pH (Sharma et al., 2018a; Haruna, 2019; Adetunji et al., 2021; Muhammad et al., 2021) likely due to the overriding influence of soil nutrient applications. Both aboveground and belowground CC biomass have been reported to significantly increase SOC by 7–17 % (Mazzoncini et al. 2011) and 30 % (Olson et al., 2014) at the top 30 cm of soil compared with NC management. Contrarily, Yang et al. (2004) and Kaspar et al. (2006) found no significant difference in SOC between CC and NC managements probably due to the time of sample collection (prior to CC termination). Further, researchers have reported that the breakdown of CC biomass residues can lead to increases in soil macronutrients (Weerasekara et al., 2017; Scavo et al., 2022). However, other researchers argue that the contribution of CCs to soil macronutrients are not discernable within the soil probably due to weather effects, CC termination timing, and nutrient recycling by subsequent CCs (Sharma et al., 2018b; Haruna and Nkongolo, 2020; Delgado et al., 2021).

Like the abiotic indicators, researchers have sought to understand the effects of CCs on the biotic indicators of soil quality, with varying results. Brennan and Acosta-Martinez (2017) reported that CCs significantly increased microbial biomass compared with NC management. Similarly, Njeru et al. (2014) and (2015) reported that CCs improved the colonization of arbuscular mycorrhizal fungi (AMF) compared with NC management. Further, (Muhammad et al., 2021) reported that total bacteria, total fungi, gram-positive (Gr+) bacteria, gram-negative (Gr-) bacteria and actinomycetes were 15, 19, 17, 11, and 23 % greater, respectively, under CC compared with NC management. Conversely, in a global meta-analysis of 60 studies, (Kim et al., 2020) reported a lack of agreement on the influence of CCs on microbial diversity due to the effects of CC termination methods (mechanical vs chemical), the quality and quantity of residues returned to the soil, differences in soil order, and climatic conditions. Therefore, these authors suggested more studies to determine the effects of CCs on soil microbial health.

The contrasts in the effects of CCs on both biotic and abiotic soil health indicators necessitates further investigations, especially in the southeastern US where there is a gap in current understanding of the effects of CCs on the biotic and abiotic soil health indicators. Further, while there are studies that evaluate the effects of CCs, and abiotic and biotic soil health indicators, there is a need for big-picture studies that combine these indicators, including soil thermal properties, across two research sites. This problem is caused by the complex interactions between these soil health indicators, and the enormous amount of work and data required for such big-picture studies. As a result, current understanding is segmented into different studies that evaluate either biotic or abiotic soil health indicators. As such, this study is novel because it reports on the big-picture effects of CCs on soil health indicators, including soil thermal properties. Therefore, the objective of this study is to a) evaluate the effects of CCs on abiotic and biotic soil health indicators, and b) assess the interaction effects of study site and CCs on soil health indicators. It is hypothesized that a) CCs will significantly improve measured soil health indicators due to their composition and diversity, and b) there will be no significant interaction effects between CCs and study site on measured soil health indicators due to differences in soil types across sites.

Table 1
Particle size distribution as a function of depth for the study sites.

	Silt (%)	Sand (%)	Clay (%)
Murfreesboro site			
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
Estill Springs site			
Depth (cm)			
0–10	22.50	63.33	14.17
10–20	21.67	61.67	16.67
20–30	20.83	63.33	15.83

2. Materials and methods

2.1. Site description

This study was conducted on two farmer fields in Tennessee, USA (Fig. 1). The first site is located in Murfreesboro (35.8176 N, −86.3737 W at 190 m asl and $a < 2$ % slope) (hereafter referred to as Murfreesboro site). The soils at this site were classified as a Cumberland silt loam (Rhodic Paleudalfs). Historically (over the last 3 decades), August (32.2 °C) is the hottest month while January (−3.7 °C) is the coldest month of the year. Additionally, May (139 mm) and October (85 mm) receives the greatest and least amount of annual precipitation, respectively. The average atmospheric temperature at the site during soil sampling was 14.2 °C.

The second site is located in Estill Springs (35.330 N, −86.012 W at 310 m asl and $a < 2$ % slope) (henceforth referred to as Estill Springs site). The soils at this site were classified as a Holston sandy loam (Typic Paleudults). Table 1 shows the particle size distributions relative to soil depth at both sites. Historically (over the last 30 years), July (31.0 ^{0}C) is the hottest month while January (−1.0 °C) is the coldest month of the

year. Further, with 122 mm and 51 mm of precipitation, the months of December and August are the wettest and driest months of the year, respectively. The average atmospheric temperature during soil sampling was 15.1 °C.

2.2. Management description

Although both sites were delineated for research during the Fall 2020, the Murfreesboro site was under 5 years of CC and 15 years of NT management previously, while the Estill Springs site was under 20 years of CC and 25 years of NT management previously. Both sites were laid out in a completely randomized design with two levels of CCs (CC vs NC) with three replicates. The tillage management at both sites was NT during the study. Each plot at both sites had a dimension of 20.1 m x 7.4 m.

The CCs of choice at the Murfreesboro site were crimson clover (*Trifolium incarnatum* L.), oats (*Avena sativa*), hairy vetch (*Vicia villosa* Roth.), winter wheat (*Triticum aestivum* L.), winter peas (*Lathyrus hirsutus* L.), flax (*Linum usitassimum* L.), triticale (*Triticale hexaploide* Lart.), and barley (*Hordeum vulgare* L.). At the Estill Springs site, the CCs were triticale, winter wheat, hairy vetch, crimson clover, oats, and cereal rye (*Secale cereale* L.). The choice of these CCs was predicated on the farmer preferences in the region. The CCs were planted in October of each year and terminated in April of the subsequent year. For this study, the CCs were first broadcasted and then drilled in during October of 2022 at both sites, and terminated during April of 2023 using about 4.2 kg ha^{-1} acid equivalent of glyphosate. The total CC biomass at Murfreesboro and Estill Springs were 1167 and 1081 kg/ha, respectively. During the study, none of the plots were irrigated, with corn (*Zea mays*) as the commodity crop. For more details on management practices at the Murfreesboro and Estill Springs sites, please see Haque et al. (2024), and Haruna et al. (2023), respectively.

2.3. Soil sampling

Soil samples were collected just before CC termination at both sites on April 24 (Murfreesboro site) and April 26 (Estill Springs site), 2023. Two sets of soil samples were collected from each plot and at each site. The first set was collected for soil physical and chemical analysis using a cylindrical core measuring 143 cm^3 in volume at 10 cm increments from the top of the soil to 30 cm deep. All excess soil samples was cut, placed in pre-labeled plastic bags, and stored in the refrigerator at 4 °C until laboratory investigation. The second set of samples were collected for the determination of microbial biomass and composition using a hand auger at the aforementioned soil depths, placed in pre-labeled plastic bags and stored in the refrigerator at 0 °C until analysis.

2.4. Physical and chemical analysis

Prior to analysis, the first set of soil samples were removed from the plastic bag, and a gauze was placed at the bottom using elastic bands. Using capillarity, the soil samples were saturated in a tub of water (electrical conductivity of 0.3 dS m^{-1} at 20 °C) for 48 hrs. Water retention analysis was conducted on intact cores at 0 kPa, −33 kPa, and −100 kPa soil water matric potentials using the methods of Dane and Hopmans (2002). At every soil water pressure, λ and C_V was determined using a KD2Pro (Decagon Devices, Pullman, WA, USA) (Zaibon et al., 2019; Haruna and Anderson, 2022). Bulk density was determined using the method of Grossman and Reinsch (2002).

Soil samples were then crushed, and passed through a 2-mm sieve. About 10 g of the <2 mm sized-particle was used to determine soil texture using the pipette method (Gee and Or, 2002). Another 10 g was used for the determination of soil pH on a 1:1 soil:water ratio by potentiometry using an electronic pH meter (Peters et al., 2015). About 250 mg of the soil was used to determine SOC and total N (TN) using the combustion method (Loss-on-ignition at 1200 °C) (Schulte and Hopkins, 1996) in a Skalar SNC (Skalar Analytical B.V., Breda, The Netherlands). Another 3 g of the soil was used for phosphate (P_2O_5, henceforth referred to as P), potassium (K), magnesium (Mg), and calcium (Ca) (using a Mehlich-3 extractant in a soil:extractant ratio of 1:5) based on colorimetric methods in a Skalar BluVision analyzer (Skalar Analytical B.V., Breda, The Netherlands) (Matula, 2010).

2.5. Microbial analysis

The second set of soil samples (collected using a hand auger) were freeze-dried using a Home Pro freeze-dryer (HarvestRight, Salt Lake City, Utah) for at least 48 hrs. The freeze-dried soils were sent for analysis at the University of Missouri Soil Health Assessment Center. The phospholipid fatty acid (PLFA) extraction procedure followed the method of Buyer and Sasser (2012). Briefly, the lipids were first removed (using Bligh-Dyer extractant:chloroform ratio of 4:1) and later segregated using solid phase extraction (SPE) in a 96-well SPE plate composed of 50 mg of silica for each well (Phenomenex, Torrence, CA). A gas chromatograph (Agilent Technologies, Wilmington, DE) controlled with MIS Sherlock™ (ver 6.3. MIDI Corp, Newark, NJ) and Agilent ChemStation software was used for lipid analysis.

Fatty acids from the PLFA analysis profiles were classified into 6 different chemical group: straight, branched, 10-methyl, cyclo, polyunsaturated fatty acid (PUFA), and monounsaturated fatty acid (MUFA). Please note that subcategories were not included in the sum for the overall category to avoid redundancy (e.g., arbuscular mycorrhizal fungi [AMF] are fungi, but the AMF marker is not included in the fungi category). Bacterial biomass was depicted by i11:0, i12:0, a12:0, 13:0, 14:0, 14:1ω9c, 15:0, a15:1, 16:0, 16:1ω9c, 17:0, cy17:0, a17:1, 18:0, cy18:0, 19:0, i20:0, and 22:0. PLFAs i11:0, i12:0, a12:0, 13:0, 14:0, 15:0, a15:1, 16:0, 17:0, a17:1, 18:0, 19:0, i20:0, and 22:0 were used to represent branched fatty acids, and these are usually linked with Gr+ bacteria. Gr- bacteria are usually related and linked with cyclopropane and MUFA (e.g., cy17:0, cy18:0, 14:1ω9c, and 16:1ω9c). Actinomycetes were depicted by 10Me16:0, 10Me17:0, 10Me17:1ω7c, 10Me18:0, 10Me18:1ωc, 10Me19:1ω7c, 10Me20:0, and 10Me22:0. PLFAs 16:3, 16:4, 18:3, 18:4, 19:3, 19:4, 20:2, 20:3, 20:5, 21:3, 22:2, 22:4, 22:5, 22:6, 23:1, 23:3, 23:4, 24:1, 24:3, and 24:4 were used to denote Eukaryotes. Fungi was depicted by 18:2, and AMF was represented by 16:1ω5c (Aliasgharzad et al., 2010; Frostegård et al., 2011; Buyer and Sasser, 2012). A complete list of PLFAs that may be identified by the MIDI software can be found in Norris et al. (2023).

Ratios were calculated for this study, and they include fungi:bacteria (F:B), predator:prey (P:P), gram-negative:gram-positive (Gr+:Gr-), saturated PLFAs:unsaturated PLFAs (sat:unsat), monounsaturated:polyunsaturated PLFAs (mono:poly), gram-metabolic status:stress (status:stress), and MUFA:branched PLFAs (MUFA:branched). The Gr+ and Gr- are included as prey, and eukaryotes are predators. The Gr- bacteria produces large amounts of MUFAs (e.g. 16:1ω7 and 18:1ω7) when metabolizing. However, most of the unsaturated fatty acid compositions are often converted to cyclo fatty acids like 17:0 cyclopropane and 19:0 cyclopropane when environmental and nutritional stress leads to a slowdown of metabolism and cell division. Therefore, a stress indicator was calculated using the methods of Villanueva et al. (2004) and Kaur et al. (2005), as:

$$Stress = \frac{(17:0\ \text{cyclo} + 19:0\ \text{cyclo})}{(16:1\omega7\text{c} + \ 18:1\omega7\text{c})} \quad (1)$$

where higher ratio values suggests greater stress. However, for the current study, the stress indicator was calculated as:

$$Stress = \frac{(16:1\omega7\text{c} + \ 18:1\omega7\text{c})}{(17:0\ \text{cyclo} + \ 19:0\ \text{cyclo})} \quad (2)$$

where a lower ratio suggests greater stress.

Table 2
Nonparametric analysis of variance of soil physico-chemical properties vs study site.

Site	BD	Volumetric water content ($cm^3\ cm^{-3}$)			0 kPa SWMP		−33 kPa SWMP		−100 kPa SWMP		SOC (%)	TN (%)	pH	P (mg kg^{-1})	K (mg kg^{-1})	Mg (mg kg^{-1})	Ca (mg kg^{-1})
		0 kPa	−33 kPa	−100 kPa	λ (W m^{-1} K^{-1})	C_V (MJ m^{-3} K^{-1})	λ (W m^{-1} K^{-1})	C_V (MJ m^{-3} K^{-1})	λ (W m^{-1} K^{-1})	C_V (MJ m^{-3} K^{-1})							
Murf	1.38a	0.44	0.33	0.32	1.66b	2.56	1.55	2.31	1.53	2.26	2.78	2.37	6.51a	42.49	779.88	13.69a	97.33a
Estill	1.31b	0.45	0.34	0.33	1.93a	2.66	1.71	2.36	1.54	2.28	2.54	2.14	5.51b	15.61	681.31	4.24b	14.15b
ANOVA $P > F$																	
Site	0.041	0.590	0.485	0.887	0.010	0.411	0.486	0.704	0.477	0.987	0.681	0.950	<0.001	0.062	0.058	<0.001	<0.001

Means with different letters within a column are significantly different at the 0.05 probability level.
Please note: SWMP = soil water matric potential; BD = bulk density; λ = thermal conductivity, C_V = volumetric heat capacity; SOC = soil organic carbon; TN = total nitrogen; P = phosphates; K = potassium; Mg = magnesium, Ca = calcium; Murf = Murfreesboro site; Estill = Estill Springs site.

Table 3
Linear mixed model showing soil physico-chemical properties vs treatment (with site as the random effect).

Site	BD	Volumetric water content ($cm^3\ cm^{-3}$)			0 kPa SWMP		−33 kPa SWMP		−100 kPa SWMP		SOC (%)	TN (%)	pH	P (mg kg^{-1})	K (mg kg^{-1})	Mg (mg kg^{-1})	Ca (mg kg^{-1})
		0 kPa	−33 kPa	−100 kPa	λ (W m^{-1} K^{-1})	C_V (MJ m^{-3} K^{-1})	λ (W m^{-1} K^{-1})	C_V (MJ m^{-3} K^{-1})	λ (W m^{-1} K^{-1})	C_V (MJ m^{-3} K^{-1})							
ANOVA $P > F$																	
Murf	0.003	0.019	0.138	0.045	0.092	<0.001	0.001	0.015	<0.001	0.451	0.744	<0.001	0.249	0.192	0.007	0.125	0.162
Estill	<0.001	0.098	0.231	0.161	<0.001	0.002	0.001	0.001	0.004	0.003	<0.001	0.088	0.346	0.276	0.244	0.181	0.199
Tmt*depth	0.613	0.318	0.712	0.587	0.470	0.791	0.699	0.865	0.592	0.985	0.444	0.356	0.837	0.421	0.241	0.557	0.820

Please note: SWMP = soil water matric potential; BD = bulk density; λ = thermal conductivity, C_V = volumetric heat capacity; SOC = soil organic carbon; TN = total nitrogen; P = phosphates; K = potassium; Mg = magnesium, Ca = calcium; Murf = Murfreesboro site; Estill = Estill Springs site; Tmt*depth = treatment X depth interaction.

Table 4
Nonparametric analysis (ANOVA) of microbial biomass and microbial biomass composition vs study site.

Site	Total microbial biomass (nmol g^{-1})	Total PLFA	AMF (nmol g^{-1})	Gr- (nmol g^{-1})	Eukaryotes (nmol g^{-1})	Fungi (nmol g^{-1})	Gr+ (nmol g^{-1})	Actinomycetes (nmol g^{-1})
Murf	57.24a	40.89a	2.36a	18.90a	0.69	0.76	11.99a	8.35a
Estill	36.47b	39.00b	1.50b	9.53b	0.79	0.99	7.32b	3.98b
ANOVA $P > F$								
Site	0.001	0.025	0.004	<0.001	0.527	0.359	0.002	<0.001

Means with different letters within a column are significantly different at the 0.05 probability level.
Please note: PLFA = phospholipid fatty acid; AMF = arbuscular mycorrhizal fungi; Gr- = gram negative bacteria; Gr+ = gram positive bacteria; Murf = Murfreesboro site; Estill = Estill Springs site.

Table 5
Linear mixed model showing microbial biomass and microbial biomass composition vs treatment (with site as the random effect).

Site	Total microbial biomass (nmol g^{-1})	Total PLFA	AMF (nmol g^{-1})	Gr- (nmol g^{-1})	Eukaryotes (nmol g^{-1})	Fungi (nmol g^{-1})	Gr+ (nmol g^{-1})	Actinomycetes (nmol g^{-1})
ANOVA $P > F$								
Murf	0.938	0.901	0.668	0.718	0.992	0.177	0.822	0.482
Estill	0.154	0.077	<0.001	0.002	0.003	0.001	0.001	0.130
Tmt*depth	0.797	0.928	0.599	0.889	0.929	0.421	0.836	0.945

Please note: PLFA = phospholipid fatty acid analysis; AMF = arbuscular mycorrhizal fungi; Gr- = gram negative bacteria; Gr+ = gram positive bacteria; Murf = Murfreesboro site; Estill = Estill Springs site; Tmt*depth = treatment X depth interaction.

2.6. Statistical analysis

The non-parametric ANOVA (Kruskal-Wallis) was conducted using proc npar1way in SAS ver 9.4 (SAS Institute, 2015) to determine any differences in soil properties at each site (soil properties vs site). Further, the effects of treatment and soil depth on soil properties and microbial biomass was analyzed using the linear mixed model (with site as the random effect). The interaction effect analyzed included treatment X depth. Statistical differences were analyzed at $p \leq 0.05$.

3. Results

3.1. Soil physicochemical properties

The non-parametric ANOVA results for soil properties vs site showed that BD, Ca, Mg, and soil pH were 5, 223, 588, and 18 % greater, respectively, at Murfreesboro site compared with Estill Springs. Conversely, λ at 0 kPa soil water matric potential was 18 % higher at Estill Springs compared with Murfreesboro (Table 2).

The linear mixed effect model results for soil properties vs treatment (with site as the random effect) showed significant treatment effects on volumetric water content (Θ) at 0 kPa and −100 kPa soil water matric potentials, λ at −33 kPa and −100 kPa soil water matric potentials, C_V at 0 kPa and −33 kPa soil water matric potentials, TN, and K. Also, there was a depth effect on BD, SOC, λ at 0 −33 and −100 kPa soil water matric potentials, and C_V at 0, −33, and −100 kPa soil water matric potentials (Table 3). There was no significant treatment X depth interaction.

3.2. Microbial biomass and community composition

The Kruskal-Wallis ANOVA test (vs site) showed that Microbial biomass and total PLFA numbers were 57 and 5 % greater, respectively, at Murfreesboro site compared with Estill Springs site. The proportion of arbuscular mycorrhizal fungi (AMF), gram negative (Gr –) bacteria, gram positive (Gr+) bacteria, and actinomycetes were 57, 98, 64, and 110 % greater, individually, at the Murfreesboro site compared with Estill Springs site (Table 4).

The linear mixed effect model results for microbial biomass composition vs treatment and depth (with site as random effect) (Table 5) showed that the proportion AMF, Gr- bacteria, eukaryotes, and Gr+ bacteria decreased with increasing depth at both sites and under both management practices (Fig. 2).

3.3. Microbial ratios

Table 6 shows the non-parametric ANOVA results for microbial ratios vs site. The F:B, P:P, Gr+:Gr-, and sat:unsat ratios were 40, 150, 9, and 43 % greater, respectively, at Estill Springs compared with Murfreesboro site. Conversely, the mono:poly, status:stress, and MUFA: branched ratios were 154, 40, and 36 % greater, respectively, at the Murfreesboro site compared with Estill Springs site.

The linear mixed effects model for microbial ratios vs treatment and depth (with site as random effect) (Table 7) showed that the F:B, status: stress, and MUFA:branched ratios decreased with increasing soil depth at both sites and under both management practices. Contrarily, the Gr–: Gr+ and sat:unsat ratios increased with increasing soil depths at both study sites and under both management practices (Fig. 3).

4. Discussions

4.1. Soil physicochemical properties

Most liquid and nutrient movement and storage, and gaseous interchange within the vadose zone are influenced by soil physical properties. To an extent, soil BD provides an estimate of the proximity between soil solid particles, and can be influenced by plant root growth. The significant differences in BD between CC and NC management could be attributed to differences in the study sites (Table 3). This management effect on BD was only significant at the Estill Springs site and was attributed to the coupled effects of CC roots and soil particle size distribution. The similarities in the root architecture of CCs at both sites suggests that the sandy loam soils at Estill Springs may have offered lower resistance to CC roots compared with the silt loam soils at Murfreesboro, and this may have resulted in the significantly lower BD values at Estill Springs site (Fig. 4).

Although Θ at saturation was similar between both study sites, it was significantly higher under CC compared with NC management at the Estill Springs site. This was probably due to the lower BD values under this treatment and at this site. However, the significantly higher Θ at −100 kPa soil water matric potential under CC compared with NC management at Estill Springs site (Fig. 4) was attributed to 1) the lower BD values, and 2) the soil texture.

The significantly higher λ at saturation at the Estill Springs site

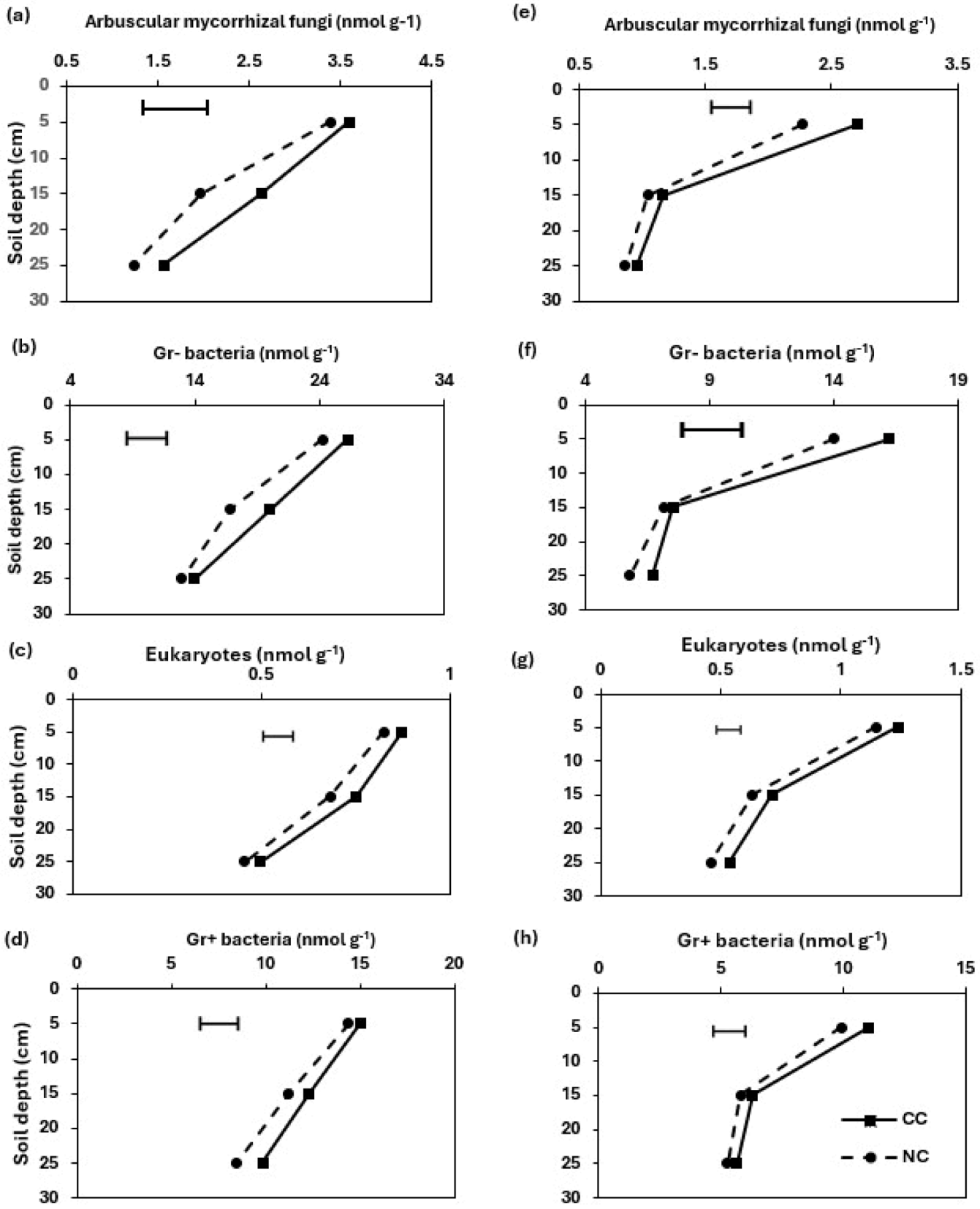

Fig. 2. Microbial biomass and composition as a function of soil depth at (a-d) Murfreesboro, and (e-h) Estill Springs research sites. Horizontal bars represent least square difference (LSD) at 0.05 probability level. Please note that Gr- = gram negative; Gr+ = gram positive; CC = cover crops; NC = No cover crop.

Table 6
Nonparametric analysis (ANOVA) of microbial ratios vs study site.

Site	Fungi:Bacteria	Predator:Prey	*G*+:G-	Sat:Unsat	Mono:Poly	Status:Stress	MUFA:Branched
Murf	0.10b	0.02b	1.13b	1.32b	12.63a	1.30a	0.94a
Estill	0.14a	0.05a	1.23a	1.89a	4.98b	0.93b	0.69b
ANOVA $P > F$							
Site	0.001	<0.001	0.066	<0.001	<0.001	0.003	<0.001

Means with different letters within a column are significantly different at the 0.05 probability level.
Please note: Gr- = gram negative bacteria; Gr+ = gram positive bacteria; Sat = saturated phospholipid fatty acids; Unsat = unsaturated phospholipid fatty acids; Mono = monounsaturated phospholipid fatty acids; Poly = polyunsaturated phospholipid fatty acids; Status = gram-metabolic status; Stress = gram-metabolic stress; MUFA = monounsaturated fatty acid; Branched = branched PLFAs; Murf = Murfreesboro site; Estill = Estill Springs site.

Table 7
Linear mixed model showing microbial ratios vs treatment.

	Fungi:Bacteria	Predator:Prey	*G*+:G-	Sat:Unsat	Mono:Poly	Status:Stress	MUFA:Branched
ANOVA $P > F$							
Tmt	0.391	0.593	0.343	0.895	0.504	0.256	0.423
Depth	0.006	0.493	<0.001	0.003	0.567	0.001	0.001
Tmt*depth	0.263	0.916	0.493	0.517	0.769	0.656	0.774

Please note: Gr- = gram negative bacteria; Gr+ = gram positive bacteria; Sat = saturated phospholipid fatty acids; Unsat = unsaturated phospholipid fatty acids; Mono = monounsaturated phospholipid fatty acids; Poly = polyunsaturated phospholipid fatty acids; Status = gram-metabolic status; Stress = gram-metabolic stress; MUFA = monounsaturated fatty acid; Branched = branched PLFAs; Tmt = treatment; Tmt*depth = treatment X depth interaction.

(Table 1) was attributed to the slightly higher Θ at 0 kPa soil water matric potential compared with Murfreesboro site since the λ of water (0.57 W m^{-1} K^{-1}) is greater than that of air (0.025 W m^{-1} K^{-1}) (Bristow, 2002). The higher water content at saturation can form a water bridge between soil particles, thus increasing λ at this soil water matric potential. Therefore, as soil moisture drains out at lower energy levels, this connectivity between the soil particles is reduced, leading to a reduction in λ. This may have resulted in the lack of significant differences in λ at other soil water matric potentials between sites. Further, the greater λ under NC compared with CC management at the Estill Springs site alone (at −33 and −100 kPa soil water matric potentials) (Fig. 5) suggests that the closer proximity of larger soil particle sizes (sandy loam soils under NC management at Estill Springs) can greatly increase thermal conductance over a temperature gradient. This is similar to the results of Alrtimi et al. (2016) and Ghuman and Lal (1985) who reported that the λ of sandy loam soils is greater than that of silt loam soils. Therefore, as the soil dries out, the λ of soil becomes more dependent on soil particle sizes and less on Θ, and this dependency is even greater under NC management compared with CC management. Similarly, the increase in BD with increasing soil depth at both sites and under both management practices may have increased λ with increasing soil depth. This further demonstrates the dependency of λ on the proximity between soil particles (Haruna et al., 2023).

Besides thermal conductance, the soil can also buffer against rapid heat change. Studies have shown that the C_V of soils is highly dependent on the Θ of the soil (the C_V of water is 4.18 MJ m^{-3} K^{-1}, compared with 2.50 MJ m^{-3} K^{-1} for SOC and 1.20 MJ m^{-3} K^{-1} for soil minerals) (Adhikari et al., 2014; Zaibon et al., 2019; Haruna et al., 2023). As such, the greater C_V at 0 and −33 kPa soil water matric potentials between CC and NC managements at Estill Springs was attributed to the differences in Θ at the study sites. Further, as the soil dries out under lower soil matric potentials, the C_V of the soils became more similar between both treatments (Fig. 6).

Total nitrogen is an indicator of soil quality and can be used as an estimate for the nitrogen availability from the decomposition of organic carbon (Li et al., 2022). Therefore, the greater TN values under CC compared with NC management at Murfreesboro site can be accredited to the numerically greater CC biomass and SOC compared to Estill Springs site (Table 2). This numerically greater SOC values, coupled with the less weathered udalfs at the Murfreesboro site (compared with the udults at Estill Springs), may have resulted in the significantly greater soil pH values at the Murfreesboro site.

In humid subtropical climates, such as the study site, soil Mg and Ca often originates from the parent materials (in the case of the study sites, mostly limestone (Abolins and Ogden, 2023)). However, the significant difference in Mg and Ca between both study sites can be attributed to the soil weathering processes as evidenced by the different soil orders present at both study sites. The Ultisols at Estill Springs, being more weathered than the Alfisols at the Murfreesboro site expectedly contained lower Mg and Ca. Since both nutrient elements are soluble, the numerically higher Θ_0, Θ_{33}, and Θ_{100} at Estill Springs site (Table 2) further agrees with this finding. Consequently, the significantly lower soil pH at the Estill Springs site can be further attributed to the lower amount of base cations at this study site.

4.2. Microbial biomass and community composition

Microbial biomass is important to SOC and nutrient transformation and is a sink and source of labile C and N (Horwath, 2017). Further, microbial biomass contains a large pool of immobilized P (Oberson and Joner, 2005) and is also a sink of soil P and critical for P transformation (Turner et al., 2013). However, microbial biomass results are not absolute, but rather an important comparative indicator of soil function with respect to differences in treatment over time within each soil. Therefore, soil microbial biomass can be an early indicator of changes in soil physicochemical properties.

The significantly greater microbial biomass and composition (specifically, AMF, Gr-, Gr+, and actinomycetes) values at the Murfreesboro site compared with the Estill Springs site can be attributed to a few reasons and mechanisms: 1) the greater BD values at this site increased the proximity between soil particles leading to more microbial proliferation (Babujia et al., 2010), 2) the lower λ (especially at saturation) which can provide optimum thermal environment over a temperature gradient (Ropelewska et al., 2016), and 3) the greater pH values due to the linear relationship between soil pH and microbial biomass composition and activities (Pietri and Brookes, 2008). Specifically, Gr+ bacteria have been reported to be chiefly responsible for the decomposition of N compounds and the amino acids bound in large organic compounds that lead to an increase in bacterial tissue (Enggrob et al., 2020). Therefore, the greater bacterial biomass at Murfreesboro site compared to Estill Springs site can also be attributed to the greater TN at the Murfreesboro site. Further, the greater bacterial biomass at Murfreesboro site can be attributed to the numerically greater SOC compared to Estill Springs sites. Therefore, the numerically greater SOC, TN, and

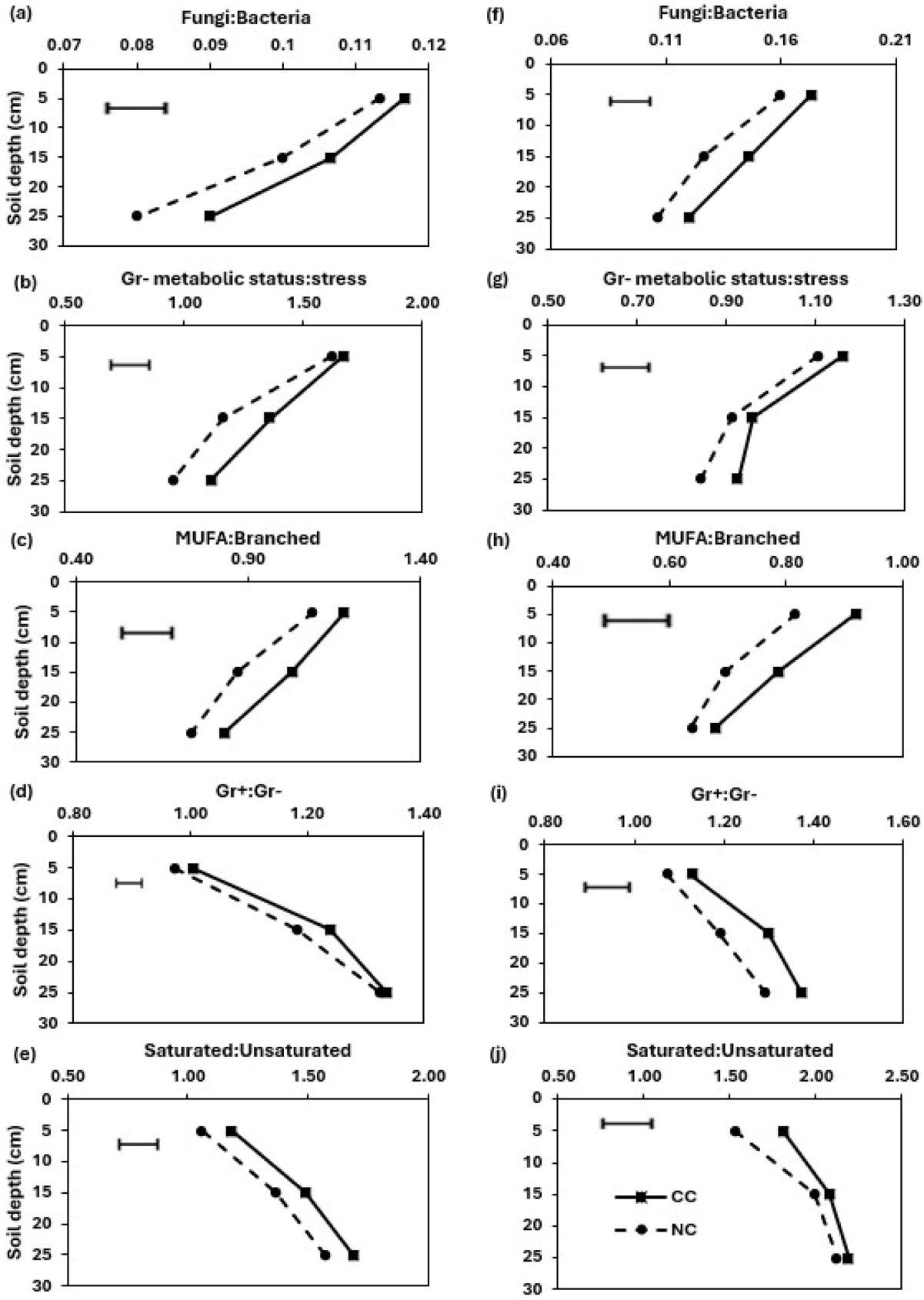

Fig. 3. Microbial ratios as a function of soil depth at (a-e) Murfreesboro, and (f-j) Estill Springs research sites. Horizontal bars represent least square difference (LSD) at 0.05 probability level. Please note that Gr- = gram negative; Gr+ = gram positive; MUFA = monounsaturated fatty acid; CC = cover crops; NC = No cover crop.

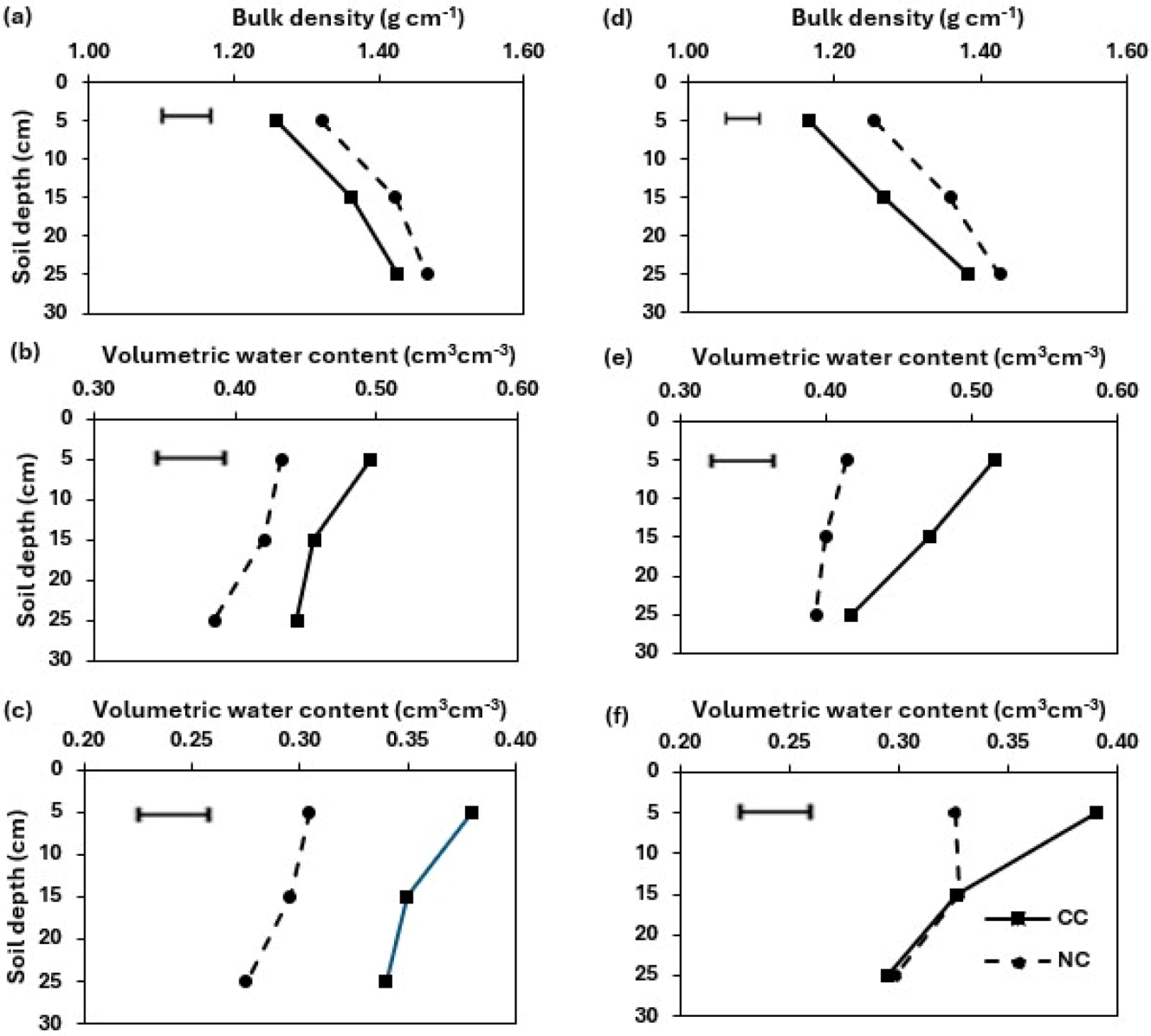

Fig. 4. Bulk density and volumetric water content at 0 kPa and −100 kPa soil water matric potentials, respectively, as a function of soil depth at (a-c) Murfreesboro site, and (d-f) Estill Springs site. Horizontal bars represent least square difference (LSD) at 0.05 probability level. Please note that CC = cover crops; NC = No cover crop.

nutrients at the Murfreesboro site can be attributed to microbial activity acting on crop biomass and resulting in nutrient transformation compared to the Estill Springs site.

The lack of treatment effects in the current study suggests that 1) 3 years of cover cropping may not be sufficient to significantly influence microbial composition and biomass, and importantly, 2) uniform nutrient application under both management practices at the beginning of the previous growing seasons may have negated the treatment effects. Therefore, longer-term (>3 years) study are needed to evaluate the effects of CCs on soil microbial biomass and composition.

It should also be noted that, although treatment effects on microbial biomass and composition was not significant, it was numerically greater under CC compared with NC management at both sites (especially at the 0–10 cm depths) and significantly decreased with increasing soil depth (Fig. 2). The CC roots may have induced a symbiotic relationship with soil microbes, and this may have resulted in the numerically greater numbers compared with NC management at both sites.

4.3. Microbial ratios

The microbial biomass ratios used in the current study are purely indices that depict relative changes in the biomass ratio and therefore do not reflect absolute biomass values. These ratios can provide valuable information on the presence or absence of soil disturbance, the health of the microbial community, nutrient cycling and decomposition, pollutant degradation, and presence or absence of other environmental stressors (Kaur et al., 2005; Mills et al., 2020).

Fungi are important decomposers (especially of lignin and hard-to-digest components of organic matter), nutrient cycling, soil aggregation, and other soil functions (Nwakanma and Unachukwu, 2017; Khan and Rao, 2019). As a result, the F:B can be an indicator of the ecosystem's capacity to self-regulate (de Vries et al., 2006). The significantly greater F:B at Estill Springs demonstrates greater breakdown of organic matter and nutrient recycling which can lead to a more sustainable agro-ecosystem with less disturbance compared with Murfreesboro site.

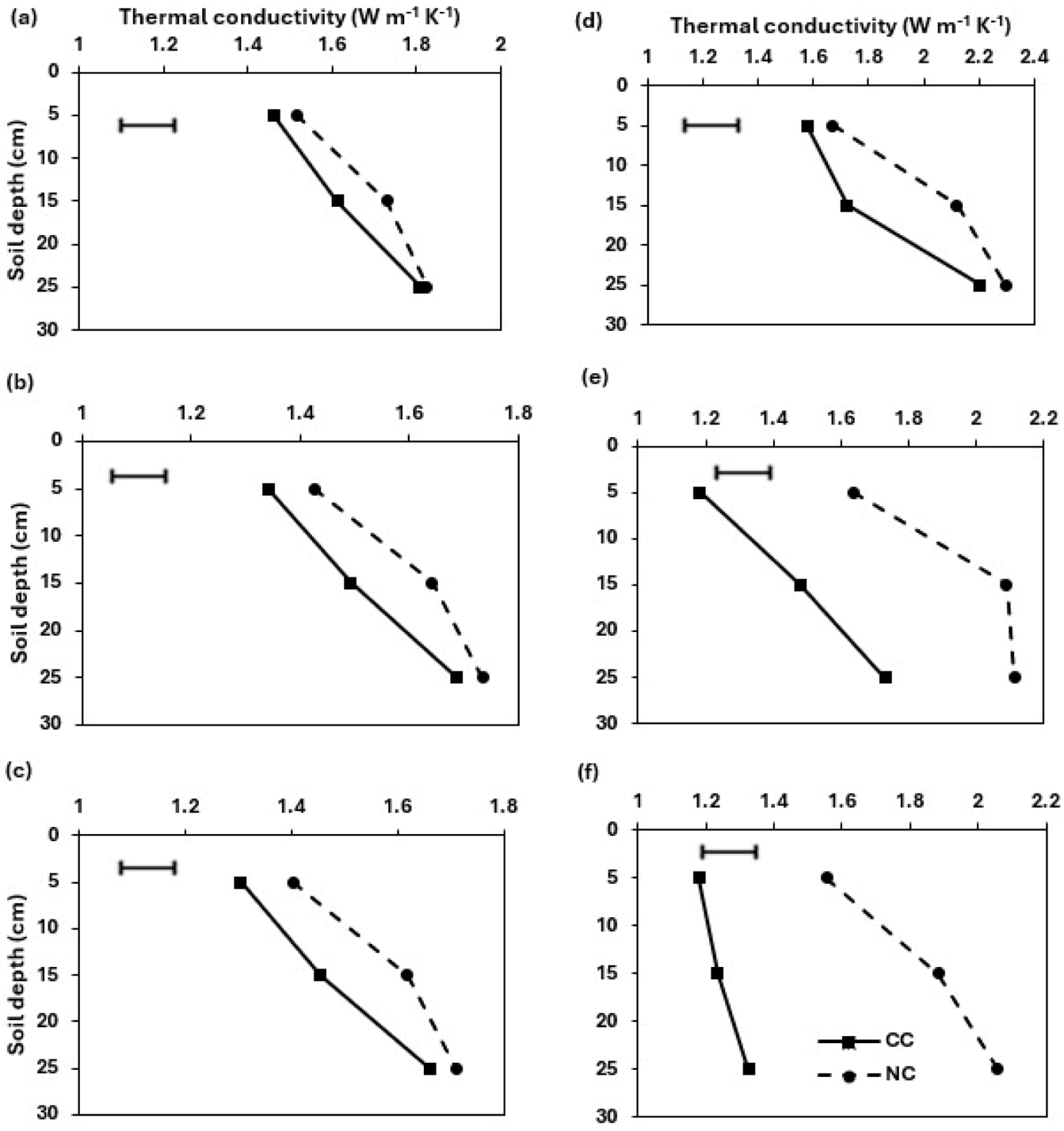

Fig. 5. Soil thermal conductivity as a function of soil depth at 0 kPa, −33 kPa, and −100 kPa soil water matric potentials, respectively, at (a–c) Murfreesboro site, and (d–f) Estill Springs site. Horizontal bars represent least square difference (LSD) at 0.05 probability level. Please note that CC = cover crops; NC = No cover crop.

This agrees with SOC and soil nutrient results in the current study. Although not significant, CC treatment had a numerically greater F:B suggesting that CC management can improve the sustainability of current cropping systems compared with NC management practices. Giusti et al. (2023) reported similar findings.

Eukaryotes are known to feed on plant residue, bacteria, and each other (Husnik and McCutcheon, 2018). Therefore, eukaryotes are classified as predators, while bacteria (Gr- and Gr+) are classified as preys for this study. A greater P:P ratio leads to more nutrient transfer within the soil ecosystem and has been related to healthier microbial communities with a greater diversity (Hohberg and Traunspurger, 2005). Consequently, the significantly greater P:P ratio at Estill Springs site suggests a more diverse soil ecosystem compared with Murfreesboro site. This agrees with results on Gr-, Gr+, and Eukaryotes from the current study. Shu et al. (2021) reported that a mix of CCs (buckwheat [*Fagopyrum esculentum*], clover, sunflower [*Helianthus annuus* L.], and radish [*Raphanus sativus*]) did not significantly improve microbial diversity compared with single CC and NC managements. Similarly, the current study had only numerical differences in microbial diversity between CC and NC (being slightly greater under CC compared with NC management) at both sites. Conversely, Vukicevich et al. (2016) reported that a mix of CCs increased microbial diversity compared with

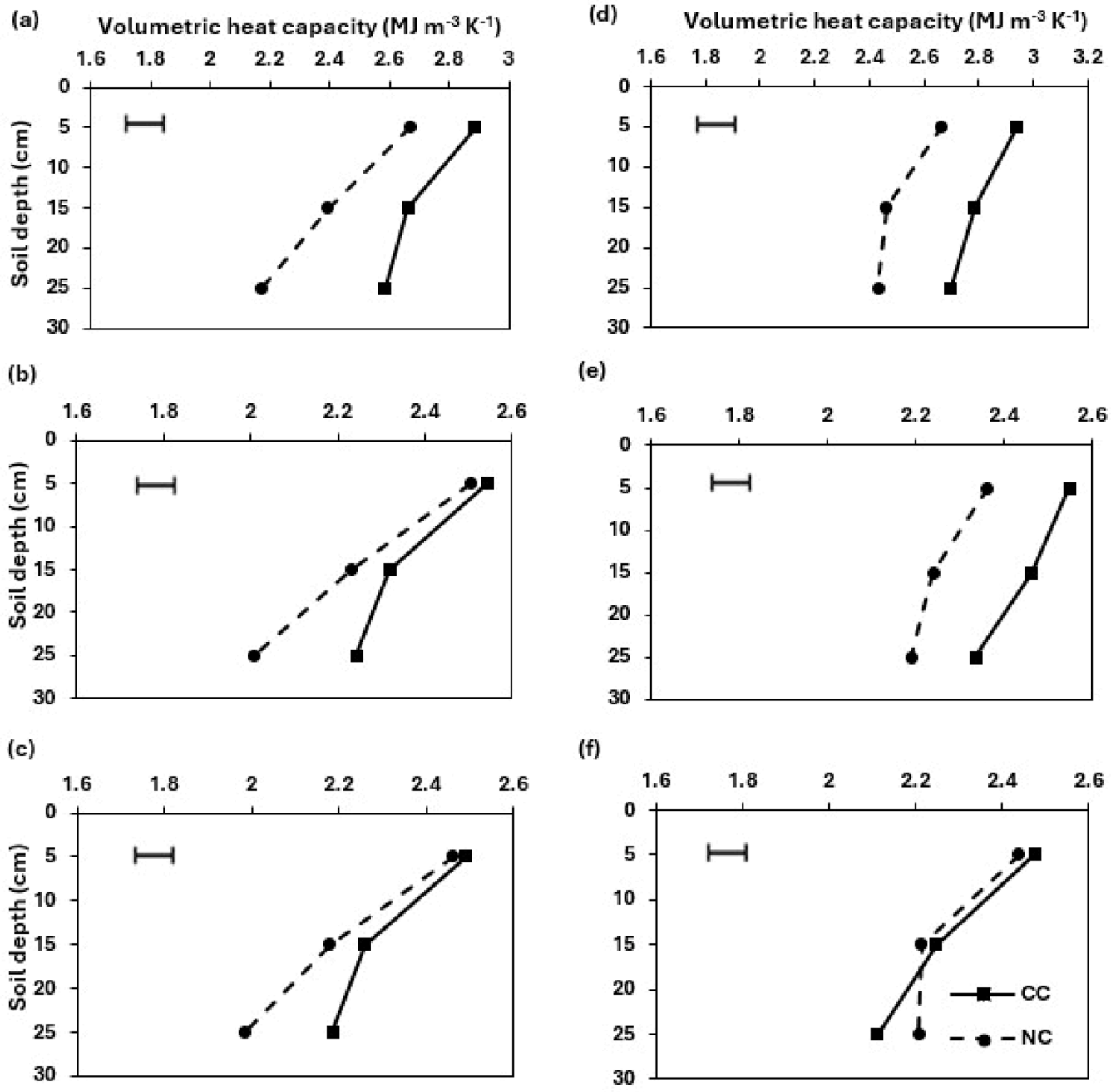

Fig. 6. Volumetric heat capacity as a function of soil depth at 0 kPa, −33 kPa, and −100 kPa soil water matric potentials, respectively, at (a–c) Murfreesboro site, and (d–f) Estill Springs site. Horizontal bars represent least square difference (LSD) at 0.05 probability level. Please note that CC = cover crops; NC = No cover crop.

NC management. The probable reason for the conflicting results seems to be the method of CC termination, since the CCs in the current study and those in the study of Shu et al. (2021) were terminated using chemicals, while those in the study of Vukicevich et al. (2016) were terminated mechanically. Cover crops terminated mechanically leads to more microbial diversity compared with herbicide use (Vukicevich et al., 2016).

Genotypic categorizations (Gr+:Gr-) and chemical structure categorization (sat:unsat, mono:poly, and Gr- metabolic status:stress) can be used as indicators of bacterial stress. Soil organic carbon is composed of a continuum of organic materials of various compositions: labile and recalcitrant. Researchers have reported that Gr+ bacteria use more C sources that are recalcitrant while Gr- bacteria use more labile plant-derived C sources (Kramer and Gleixner, 2008). Since both groups depend on different sources of C in the soil, a proportional shift in their presence can be used as an indicator of energy constraints in soils, or stressful soil conditions such as low gaseous interchange, suboptimal pH, low nutrient supply, or heavy metal contamination (Pennanen et al., 1996). The current study demonstrates that the Ultisols at the Estill Springs site have more relative C availability for microbial communities compared with the Alfisols at the Murfreesboro site. This may have also partly resulted in the more diverse microbial population as predicted by the P:P ratio.

Previous studies have linked greater sat:unsat, and lower mono:poly and Gr- metabolic status:stress ratios with more stress in bacterial populations (Moore-Kucera and Dick, 2008; Drenovsky et al., 2010; Francisco et al., 2016). The results of the current study shows that bacterial populations were under more stress at the Estill Springs site

compared with the Murfreesboro site. This can be attributed to 1) the slightly greater population of predators (eukaryotes) (as shown in the P:P ratio results), 2) the lower TN values, and 3) the slightly more acidic conditions at the Estill site compared with the Murfreesboro site. As a result, microbial population was lower at the Estill Spring location, and this can have negative implications on residue decomposition and pollutant breakdown.

Monounsaturated fatty acids are generally produced by AMF, Gr-, and fungi, while PUFAs are produced by micro eukaryotes. The MUFA:PUFA ratio further show a shift towards Gr- bacteria at the Murfreesboro site and more shift towards predators and, consequently, more microbial diversity at the Estill springs. Further, Zhang et al. (2015) and Zhang et al. (2018) suggested that the MUFA:PUFA ratio can be used as indicators of the proportion of aerobic to anaerobic microorganisms. As such, this suggests a slightly more anaerobic condition at the Estill Springs site compared with the Murfreesboro site, and this is supported by the soil physical properties results in the current study.

In general, results disproved the first hypothesis of this study but agreed with the second hypothesis. Although the current study did not show significant differences between soil management practices, it still showed some numerical differences between CC and NC management practices at one or both sites. Most microbial markers suggests that CC management can improve microbial activity and diversity compared to NC management, especially at the Estill Springs site. Although not the major goal of this study, it is quite interesting to see the differences in soil properties between the two soil orders in the current study. Future studies should investigate the interactive effects of management practices and soil orders on microbial activity and diversity. This will provide more insight into region- and soil-specific management practices for improved soil health.

In the current study, there does not appear to be any significant effect of laboratory-measured soil thermal properties on microbial activity and diversity. Another recommendation for future studies is the investigation of the effects of in-situ measured soil thermal properties on microbial activities and diversity. This may provide insight on the effects of climatic variability on microbial functions.

5. Conclusion

This study investigated the effects of cover crops on the coupled physico-chemical and biological properties of the soil. Results were mixed, with some significant positive effects of CCs on some soil properties and no significant effect on others. Further, there was very minimal effects of CCs on microbial biomarkers, suggesting that the three-year duration of this study may not be sufficient for noticeable effects of this management practices on soil microbial activity. There is, therefore, a need for further study, especially on the effects and relationship between field-measured soil properties and microbial activity, not just prior to CC termination, but during the growing season.

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CRediT authorship contribution statement

Samuel I. Haruna: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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.

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Data availability

Data will be made available on request.

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