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Short-term effects of Kernza and alfalfa on microbial communities

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Abstract

Continuous land disturbance could negatively impact microbial community, but perennial crops can potentially reverse this negativity. The objective of this study was to evaluate the effects of Kernza (*Thinopyrum intermedium*) and alfalfa (*Medicago sativa* L.) on soil microbial structure and stress condition using the phospholipid fatty acid profiling. The study was conducted at the Ross Jones Research Farm, University of Missouri and consisted of four treatments: Kernza fertilized, Kernza unfertilized, Kernza and alfalfa intercrop, and alfalfa monocrop with four replications. Treatments were established in September 2021 on 18.3 m × 18.3 m plots. Soils from 0- to 5-cm and 5- to 15-cm depths were sampled in September 2021 (before treatments were placed) and 2022 and analyzed for microbial communities. All microbial communities increased after 1 year with the perennial crops. Since differences were not significant among treatments in 2022, this may lead to positive impacts of perennial crops on microbial communities, irrespective of the crop species and management. Moreover, community structure modifications were also observed with the perennial crops, irrespective of the species and management, as evidenced with changes in bacterial community indices in 2022. While fungi/bacteria ratio increased, Gram-positive/Gram-negative bacteria ratio decreased in 2022, suggesting a reduction in microbial stress, which can be attributed to ecological functions of the perennial crops. The study showed improvements in soil microbial biomass and modifications in microbial community structure after 1 year of Kernza and alfalfa. As the system matures, relative benefits of management (fertilization and intercropping) and plant species may be realized.

Abbreviations: AM, alfalfa monocropping; BB, bacterial biomass; CEC, cation exchange capacity; FB, fungi biomass; IWG, intermediate wheatgrass; KA, Kernza and alfalfa intercrop; KF, Kernza fertilized; KU, Kernza unfertilized; PLFA, phospholipid fatty acid; PLS-PM, partial least square path model; SOC, soil organic carbon; TMB, total microbial biomass.

1 | INTRODUCTION

Unsustainable agricultural activities are impacting ecosystem services including soil health indicators, water quality, and land productivity (Al-Kaisi, 2008; Montgomery, 2007; Udawatta et al., 2019). Global land degradation annual cost is $231 billion per year (Baumgartner & Cherlet, 2015), and soil loss rate in the United States is about 2 mm annually (Thaler et al., 2022). Forty-four rivers and streams and 64% of lakes reservoirs are impaired and unsuitable for human use and recreational activities (USEPA, 2017). Thus, the major cause of water pollution is agriculture, as evidenced with the previous outcomes. Corn–soybean watersheds annually lose 13–30 kg N ha^{-1} and 0.29–3.59 kg P ha^{-1} (Udawatta et al., 2004, 2006; USDA-NRCS, 2013). If all these nutrients are retained in their respective areas, hypoxia of Gulf of Mexico may not occur, fertilizer application rate will decline, and fossil fuel use for fertilizer manufacturing and applicators will reduce.

Improvements in soil, water, and nutrient conservation can be achieved by implementation of various conservation practices, including a continuous living cover. For instance, sheet and rill erosion on crop lands estimated by the Revised Universal Soil Loss Equation in the United States was decreased from 1.7×10^9 t $year^{-1}$ in 1982 to 960×10^6 t $year^{-1}$ in 2007, a 43% reduction (USDA, 2011). Soil and nutrient losses from fields are reduced by the continuous living covers while improving soil health as soil disturbance is minimized. Other benefits of perennial species such as the grain Kernza and alfalfa over annual crops include improvements in soil porosity, infiltration, water storage, nutrient retention, biodiversity, and reduced erosion (Alagele et al., 2019, 2020; Chamberlain et al., 2022; Crews & Brookes, 2014; Crews et al., 2016; Seobi et al., 2005; Udawatta et al., 2008), which help enhance soil quality and land productivity. Additionally, soils with a living cover can serve as a buffer for temperature and water fluctuations, thus developing more stable and favorable conditions for soil organisms, carbon (C) storage, and soil–water relations (Adhikari et al., 2014; Mendis et al., 2022).

Perennial grains have been promoted in recent years due to their multiple benefits like potential to address system diversification, environmental, and production challenges (Duchene et al., 2019). Kernza is the trademark name for the grain of *Thinopyrum intermedium*, an intermediate wheatgrass (IWG) being developed at The Land Institute, Kansas (Coyne, 2022), and it is a promising deep-rooted forage grain crop (DeHaan & Ismail, 2017). This is the only known perennial grass species in the whole world that can serve two purposes: forage for livestock and grains for humans (Coyne, 2022). Since it is a perennial grain species, annual replanting is not required, resulting in reduced soil disturbance and erosion (DeHaan et al., 2023). Continuous living cover, greater C uptake (Duchene et al., 2019), improved soils, and no disturbance can create favorable conditions for soil communities and crop growth. Therefore, IWG can enhance the structure, abundance, and the composition of soil microbes. Further, conversion from annual to perennial grains could potentially store up to 1.7 t SOC ha^{-1} in year 1 (Crews & Rumsey, 2017), suggesting the long-term potential of IWG as a carbon sink (Oliveira et al., 2018). The root systems of perennial crops also support greater nutrient recycling compared to annual crops. According to Sainju et al. (2017), IWG and other perennial grasses had 12–16 times greater root mass, carbon, and nitrogen (N) than annual grain wheat.

> **Core Ideas**
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> - Perennial crops, irrespective of the species, enhanced phospholipid fatty acid microbial communities after 1 year of establishment.
> - Benefits by plant type may not be observed in the short term.
> - Long-term Kernza and alfalfa establishments may favor enhanced soil health.

Alfalfa is a perennial leguminous forage crop widely grown in the United States, and the average yield per ha in the US central Great Plains is 7.6 Mg (Fink et al., 2022). As a leguminous crop, it has the potential to fix N with the help of a nitrogen-fixing symbiont bacteria, and about 1 billion kg of N per year is fixed by alfalfa, accounting for one-fifth of the total amount of N fertilizer applied to all crops (Peterson & Russelle, 1991). Thus, the crop is preferable for rotational cropping or intercropping with non-nitrogen fixing cereal crops such as corn and wheat, which usually require N fertilization to give optimal grain yield (Ma et al., 2023). Being a perennial crop, alfalfa is also recognized for its adaptation to drought and seasonal weather variabilities.

Among the various soil microbial quantifications, phospholipid fatty acid (PLFA) profiles are strongly related to soil quality (Bossio et al., 1998). PLFA profiles have differentiated microbial communities between crop rotations, tillage, residue management, cover crops, buffer strips, fertilizer applications, soils, depths, seasons, landscapes, and system maturity (Alagele et al., 2020; Arcand et al., 2016; Bossio et al., 1998; Fierer et al., 2007; Hamel et al., 2006; Mbuthia et al., 2015; Nivelle et al., 2016; Rankoth et al., 2019). The community ratios such as fungi to bacteria and Gram-positive/Gram-negative bacteria (GP/GN) have been widely used to trace microbial communities' stress condition, mainly referred to as lack of sufficient moisture or nutrients (Agnihotri et al., 2023; Cheng et al., 2024). However, PLFA profiling for microbial communities under Kernza and alfalfa is limited. The objectives of this study were to (1) evaluate

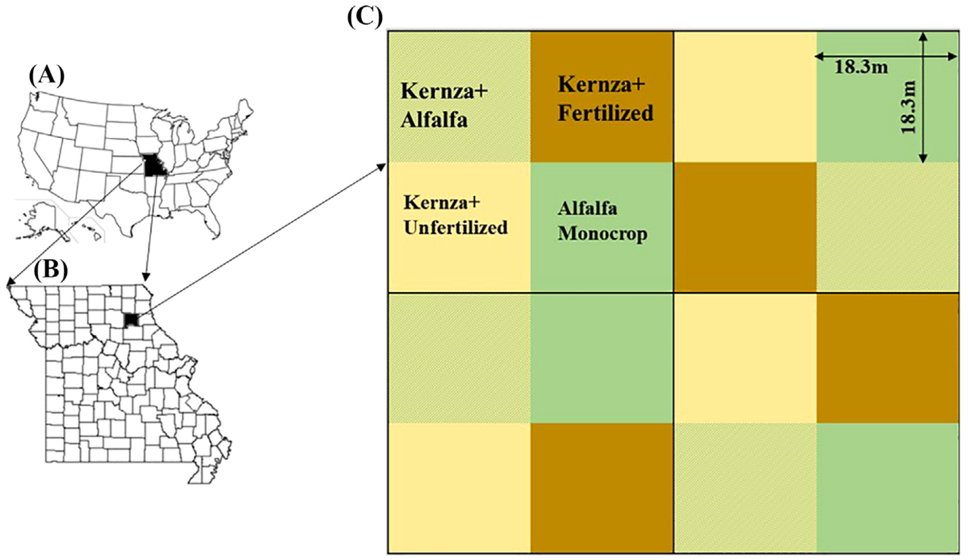

FIGURE 1 Location of the study area (A: USA; B: Shelby County [shaded dark] in Missouri State; C: experimental field layout).

soil microbial community structure and ratios and (2) understand the relationships between soil organic carbon (SOC) and PLFA microbial community profiles under Kernza, alfalfa, and Kernza and alfalfa intercrop treatments.

2 | MATERIALS AND METHODS

2.1 | Study site, management, and experimental design

The study was conducted at the Ross Jones Research Farm, northeast Missouri (39˚57′ N and 92˚03′ W; Figure 1). The experimental site had nearly zero slope. The soil at the site is Mexico silt loam (fine, smectitic, and mesic Vertic Epiaqualfs) and formed in loess over loamy sediments derived from till (Unklesbay & Vineyard, 1992). The soil parent materials are glacial till and windblown Peorian loess. These soils have a claypan subsoil layer, and therefore, drainage is poor and classified in hydrologic group D. The climatic condition of the study area is presented in Figure 2. The area received greater annual precipitation in 2021 (1091 mm) compared to 2022 (765 mm). The precipitation of 2022 was approximately 20% below the long-term average of 963 mm. On the other hand, the air temperature pattern remained consistent across both sampling years and aligned with long-term average values. The average long-term annual air temperature is approximately 11.7˚C, with an average monthly low of −6.6˚C in February and an average monthly high of 31.4˚C in July. The snowfall is about 590 mm year^{-1} and can stay on the ground for extended periods (Missouri Climate Center, 2022).

Previously, the site was under alley cropping practice with a corn (*Zea mays* L.)–soybean [*Glycine max* (L.) Merr.] rotation until 2017. Eight rows of silver maple (*Acer saccharinum* L.) saplings (1-0 bare-root stock) were planted in 1990 to create 20-m wide crop alleys for the crop rotation, and the trees were thinned in 1996 (Miller & Pallardy, 2001). Trees were removed in 2017, and the site was converted to a corn–soybean rotation until the current project was established in September 2021. It was under soybean crop in 2021. Before seeding, soybean stubble was removed, and soil was tilled with a vertical till for approximately 10-cm depth. Kernza and alfalfa were seeded at 17.7 and 31.8 kg ha^{-1} rates, respectively, on September 9, 2021.

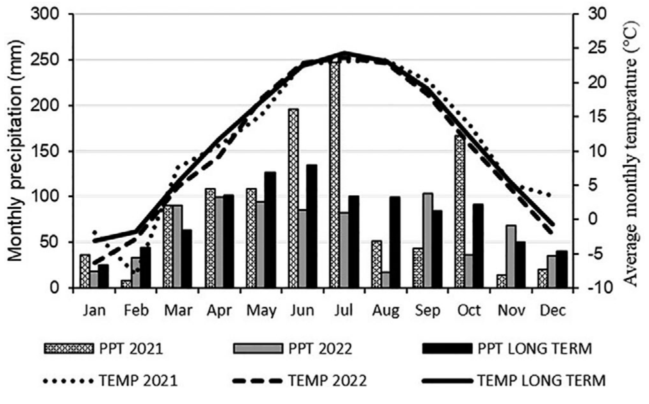

FIGURE 2 Monthly rainfall (PPT) and mean monthly temperature (TEMP) for the study years 2021 and 2022, compared with the long-term (2001–2022) mean monthly rainfall and mean monthly temperature.

The study design was randomized complete block design (RCBD) with four blocks, four treatments, two depths, four replications, and presence or absence of intervention (years: 2021 and 2022). Treatments were Kernza fertilized (KF), Kernza unfertilized (KU), Kernza and alfalfa intercrop (KA), and alfalfa monocropping (AM).

2.2 | Soil sampling and analysis

A grid sampling was conducted in September of 2021 (before treatments were established) and 2022 (after one year with the treatments). Per plot, sampling was conducted at four random grid points. Accordingly, four samples per each depth (0–5 and 5–15 cm) were collected. These were thoroughly mixed and put in Ziplock bags, forming one composite sample per plot per depth. Then, each sample was kept in a coolant until brought to Soils Lab, University of Missouri Columbia. About 50 g portion of each sample was kept in refrigerator until sent to Wards Lab (https://www.wardlab.com/) in Nebraska for PLFA profiling. The remaining portion of each of the 2021 samples was air-dried, crushed, and removed off roots for texture and chemical properties. The remaining portion of each of the 2022 samples was handled similarly but analyzed only for SOC content. Texture, pH_{salt}, major cations (Ca [calcium], Mg [magnesium], and K [potassium], by ammonium acetate extraction), cation exchange capacity (CEC; Nathan et al., 2012; Woodruff, 1948), and SOC (loss on ignition method; Ball, 1964) were determined at the Soil Testing Laboratory, University of Missouri (Nathan et al., 2012).

2.3 | PLFA analysis

Two surface soil depths were analyzed for microbial communities by the PLFA procedure for both sampling years at Ward Laboratories, Nebraska, according to standard procedures (Ward Laboratories Inc., 2020). This procedure includes five main steps, such as extraction, lipid-class separation, fatty acid methyl ester extraction, gas chromatography analysis, and peak separation/identification. Frozen (2 g) soils were shaken with dichloromethane (DMC, CH_2Cl_2):methanol (MeOH):citrate buffer (1:2:0.8 v/v/v) in test tubes for 1 h at 240 rpm for total soil lipid extraction. Samples were shaken again with 2.5 mL of DMC and 10 mL saturated KCl solutions, then centrifuged, and organic fraction was pipetted into vials. These samples were dried in nitrogen, dissolved in DCM, and stored at −20˚C.

Lipid-class separation was done with Silica gel columns; samples were loaded onto columns, and vials were washed twice with small amounts of DCM. Then neutral, glycolipid, and phospholipid fractions were eluted by sequential leaching with DCM, acetone, and methanol, 2 mL of each, respectively. The phospholipid fractions were collected in 4-mL vials after discarding the neutral and glycolipid fractions. These samples were dried in nitrogen, dissolved in MeOH, and stored at −20˚C.

Acid methanolysis was used to extract fatty acid methyl esters. MeOH/H_2SO_4 (25:1 v/v) was added to the vials using a pasteur pipette and placed in an oven at 80˚C for 10 min. Vials were vortexed for 30 s and left to settle for 5 min after 2 mL of hexane (C_6H_{14}) was added at the room temperature. The lower fraction was discarded, and the rest was dried in nitrogen at 37˚C in a fume hood. A total of 100 µL of hexane was added and vortexed to prepare for gas chromatograph (GC) analysis.

Samples were analyzed on GC and MIDI's Sherlock software systems. Agilent 7890A GC (Agilent Technologies) equipped with a 7693 autosampler and a flame ionization detector (FID) analyzed samples. The hydrogen was the carrier gas at 30 mL min^{-1}, and the column was a 50-m Varian Capillary Select FAME # cp7420. Two microliter of sample was injected in 5:1 split mode. FID was at 300˚C and the injector at 250˚C. The initial oven temperature of 190˚C was raised to 210˚C (2˚C min^{-1}), then to 250˚C (5˚C min^{-1}), and held for 12 min.

Peaks were differentiated by comparing retention times of known standards (Supelco Bacterial Acid Methyl Esters #47080-U, plus MJS Biolynx #MT1208 for 16:1ω5). A range of concentrations of 19:0 FAME standards dissolved in hexane was used to develop standard curves and determine PLFA quantities. The PLFA amounts were expressed in ng PLFA g^{-1} dry soil.

2.4 | Data analysis

A three-way analysis of variance (ANOVA) was employed to understand effects of treatments (KF, KU, AM, and KA), intervention (before and after intervention: 2021 and 2022), and soil depth (two: 0–5 cm and 5–15 cm) on each PLFA microbial community (fungi, actinomycetes, bacteria, etc.) and community ratios, for example, the GP/GN ratio. It was conducted in R-studio using the Agricolae package (Mendiburu & Yaseen, 2022) as RCBD, as described by Steel et al. (1997). Observations for each variable were checked for normality using the Shapiro–Wilk's method (Shapiro & Wilk, 1965) and validated for the assumptions of ANOVA using the Bartlett's test (which assumes data are normally distributed), before a two-way repeated ANOVA was conducted. Fisher's least significance difference (LSD) was used to compare means across treatments, intervention regimes (with and without perennials), or depth using the "LSD test" function in the Agricolae package.

Treatment, intervention, and depth were fixed effects. Random effects were replications. In addition, a principal component analysis was conducted on microbial community data, microbial community ratios, and stress ratios (saturated/unsaturated fatty acids [Sa/Unsa FAs] and monounsaturated/polyunsaturated fatty acids) to understand how these parameters alone could capture the variabilities due

TABLE 1 Summary (mean ± SD) of soil parameters for the study site in 2021 (before treatments were established).

Depth	Parameter	AM	KA	KF	KU	*p* value
0–5 cm	pH	6.0 ± 0.2	6.0 ± 0.3	6.0 ± 0.2	6.0 ± 0.4	0.855
	SOC (%)	2.04 ± 0.16	2.09 ± 0.17	2.02 ± 0.27	2.12 ± 0.25	0.442
	P (kg/ha)	60.1 ± 26.4	75.2 ± 31.7	46.6 ± 8.6	57.9 ± 20.4	0.019
	Ca (kg/ha)	4531.04 ± 490.04	4573.5 ± 610.9	4468.7 ± 303.0	4591.5 ± 639.8	0.936
	Mg (kg/ha)	346.5 ± 38	344.7 ± 41.1	340.2 ± 26.2	338.7 ± 37.8	0.926
	K (kg/ha)	430 ± 102.9	521.8 ± 178.1	371.2 ± 30.3	407.1 ± 76.9	0.004
	CEC (cmol [+]/kg)	13.5 ± 1.2	13.7 ± 1.2	13.0 ± 0.8	13.7 ± 1.4	0.506
	Sand (%)	13.1 ± 3.1	12.2 ± 2.9	13.9 ± 2.2	12.3 ± 3.9	–
	Silt (%)	70.2 ± 2.8	70.9 ± 3.4	69.1 ± 2.6	70 ± 3.7	–
	Clay (%)	16.7 ± 1.2	16.9 ± 1.7	17 ± 1.4	17.7 ± 2.8	–
5–15 cm	pH	6.3 ± 0.3	6.2 ± 0.5	6.3 ± 0.4	6.3 ± 0.5	0.962
	SOC (%)	1.54 ± 0.2	1.63 ± 0.15	1.56 ± 0.16	1.69 ± 0.24	0.226
	P (kg/ha)	20.9 ± 9.6	35.2 ± 18.2	15.8 ± 5.9	25.1 ± 13.2	0.001
	Ca (kg/ha)	4870.1 ± 492.3	4763.2 ± 686.4	4823.2 ± 483.5	4814.3 ± 671.3	0.961
	Mg (kg/ha)	311 ± 39.3	304 ± 42	295.6 ± 34.1	291.5 ± 41.9	0.493
	K (kg/ha)	244.2 ± 44.9	308.1 ± 102.2	209.4 ± 32.3	257.3 ± 68.9	0.003
	CEC (cmol [+]/kg)	13.3 ± 1.0	13.4 ± 1.4	13.1 ± 1.1	13.2 ± 1.2	0.851
	Sand (%)	11.6 ± 2.7	11.4 ± 2.9	12.7 ± 4.5	10.9 ± 2.6	–
	Silt (%)	70.3 ± 2.2	70.2 ± 3.4	69.2 ± 4.7	69.8 ± 3.8	–
	Clay (%)	18.3 ± 1.5	18.4 ± 2.2	18.1 ± 1.7	19.2 ± 2.5	–

Abbreviations: AM, alfalfa monocrop; CEC, cation exchange capacity; KA, Kernza and alfalfa intercrop; KF, Kernza fertilized; KU, Kernza unfertilized; SOC, soil organic carbon.

to treatment, depth, and management. Furthermore, we also conducted a partial least square path model (PLS-PM) to understand the relationships between SOC and microbial community structures using the "plspm" package in R (Sanchez, 2013).

3 | RESULTS AND DISCUSSIONS

3.1 | Characteristics of the soils before treatments established

Soil properties before Kernza or alfalfa were planted are summarized in Table 1. Soils were slightly acidic with a mean pH of 6.0, which was slightly lower than the dominant soil pH levels (>6.1) of the Shelby County (Nathan et al., 2007). The texture was silty loam. Available P ranged from 46.6 ± 8.6 to 75.2 ± 31.7 kg ha^{-1} in the topsoil layer, and it was much lower (up to 80%) in the lower depth. CEC was similar across the two depths, with values ranging from 12.9 ± 1.2 to 13.4 ± 1.4 cmol kg^{-1} soil. The mean organic matter content in the top depth was 3.6% and decreased to less than 3% in the subsequent layer, resulting in up to 30% decrease in the 5- to 15-cm layer compared to the 0- to 5-cm depth. It was only the top 0–5 cm that had a mean SOC greater than the minimum critical range, which is ~2% (Loveland & Webb, 2003), suggesting the overall soil organic matter in the study site was considerably low.

3.2 | Kernza and alfalfa effects on SOC

There was a significant decline in SOC content in the upper 0–5 cm under all the treatments after 1 year with the crops (Figure 3). The loss was similar for all the treatments, except KU, which had the greatest SOC content at this depth in 2022. The decrease was 15% for KU and 20%–25% in the other treatments. The loss can be mainly attributed to the disturbance due to the tillage carried out before seeding the treatments (Ogle et al., 2005). It can also be due to the priming effect of the rhizosphere of the newly introduced plants (Dijkstra & Cheng, 2007) that may have increased microbial activities, which in turn may have triggered SOC turnover. A previous global assessment of SOC changes after the conversion of annual crops to perennial crops by Ledo et al. (2020) also revealed the possibility of a temporary decline in SOC content. A recent meta-analysis by Siddique et al. (2023) further underscored that short-term changes following such conversions are often a decline rather than an increment, suggesting it could take up to 5 years with the perennials to see the

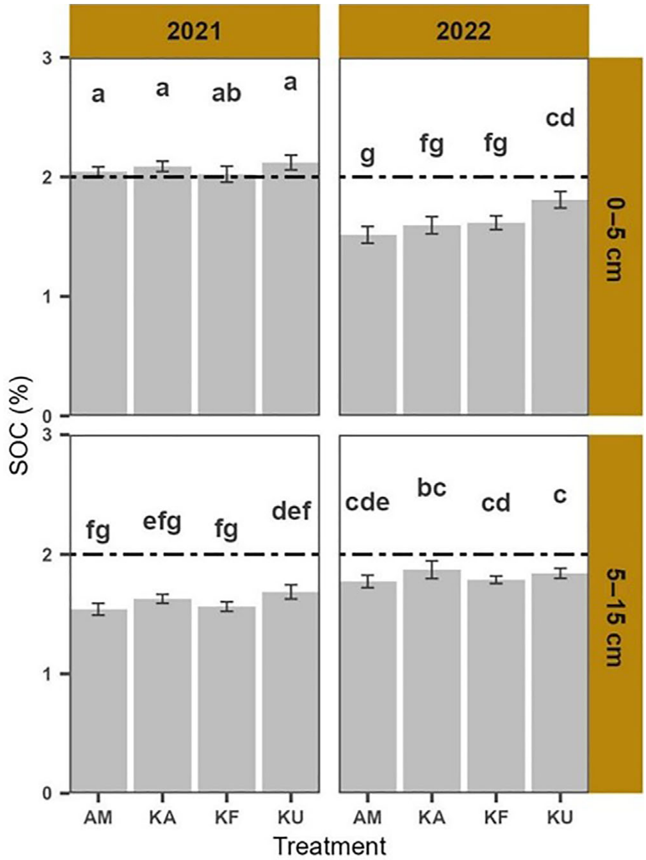

FIGURE 3 Post hoc test results showing mean comparisons of soil organic carbon (SOC) content by depth and year. Bars (mean ± SE) sharing the same letter do not differ ($p > 0.05$). AM, alfalfa monocrop; KA, Kernza and alfalfa intercrop; KF, Kernza fertilized; KU, Kernza unfertilized.

benefits over the previous annual crops in terms of the soil carbon storage. However, such impacts may be limited to only the surface layer (0–5 cm depth).

In contrast the trend in the surface layer, SOC content increased in the sub-surface layer (5- to 15-cm depth) in year 2022 for each treatment, although the differences were not significant among treatments. The mean values in percentage in the year 2022 were 1.5%, 1.6%, 1.7%, and 1.8%, and the increment compared to year 2021 was 15%, 14%, 15%, and 9% for AM, KA, KF, and KU, respectively. Generally, it was low, irrespective of treatments, depth, or year. It was mostly below 2%, known as the "critical level" (Loveland & Webb, 2003), below which soils may lack the capacity to support proper ecosystem functions.

3.3 | Effects on microbial community structure

The results of microbial community are summarized in Figure 4. Total microbial biomass (TMB), fungi biomass (FB), and bacterial biomass (BB) significantly increased after 1 year (2022) with the perennials, although differences among the treatments were not significant ($p > 0.05$) for all the PLFA microbial community profiles. Similar patterns were observed for bacterial and fungi subgroups (Figures S1 and 2). In the year 2021, before treatments were placed, differences were observed between depths and among the treatments' locations. The results of year 2021 were not related to any effects of the treatments, and the specified results indicated with each of the treatments in this year were only to showcase the variabilities in the PLFA profiles among the plots allocated to the treatments.

The results showed that all treatments performed well in terms of supporting microbial communities, and the microbial community was similarly affected. This contrasts with a previous report by Finney et al. (2017), who found different levels of impacts of cover crops on soil microbial communities, indicating some cover crop species greatly favor microbial communities than some other species. However, these were not perennials, unlike those in the current study, which may take years to observe species-level impacts. McKenna et al. (2020) evaluated effects of annual crops and perennial crops' (including Kernza) on fungi communities and found that there was no difference among the different types of perennials, although greater diversity was observed with the perennials compared to the annual crops.

The mean TMB ranged from 630 to 1597 ng g^{-1} of soil in 2021, and TMB was increased by >fivefold (3490–5200 ng g^{-1}) after 1 year of establishment, regardless of the type of the treatments applied. Similar trends were observed for FB and BB, despite BB being generally greater than FB. It increased by >eightfold for FB and was 14–96 ng g^{-1} in 2021 and 416–762 ng g^{-1} in 2022, whereas for BB, it ranged from 180–539 ng g^{-1} in 2021 to 1585–2365 ng g^{-1} in 2022. These results show that the possible shift in soil microclimate under these perennial crops may have triggered microbial growth. However, any relative importance or specific role related to the plant species (alfalfa and Kernza) and management (e.g., fertilization and non-fertilization) was not reflected. Although belowground diversity and biomass are very well correlated with above ground plant species (Eisenhauer et al., 2010), the results of the current study did not show any relationship between belowground microbial biomass and plant species. The significant growth in microbial abundance after 1 year with the perennials shows that a shift in soil microbial structure can be possible in just 1 year. However, it also suggests that the system needs more time to establish at the site and species to mature before differences can be detected between these selected species (Gurmessa et al., 2021). A previous study by Chamberlain et al. (2022) also showed a rapid rise in soil microbial biomass under 1-year-old perennial crops, including a perennial wheat. However, in contrast to our findings, these authors highlighted mixed

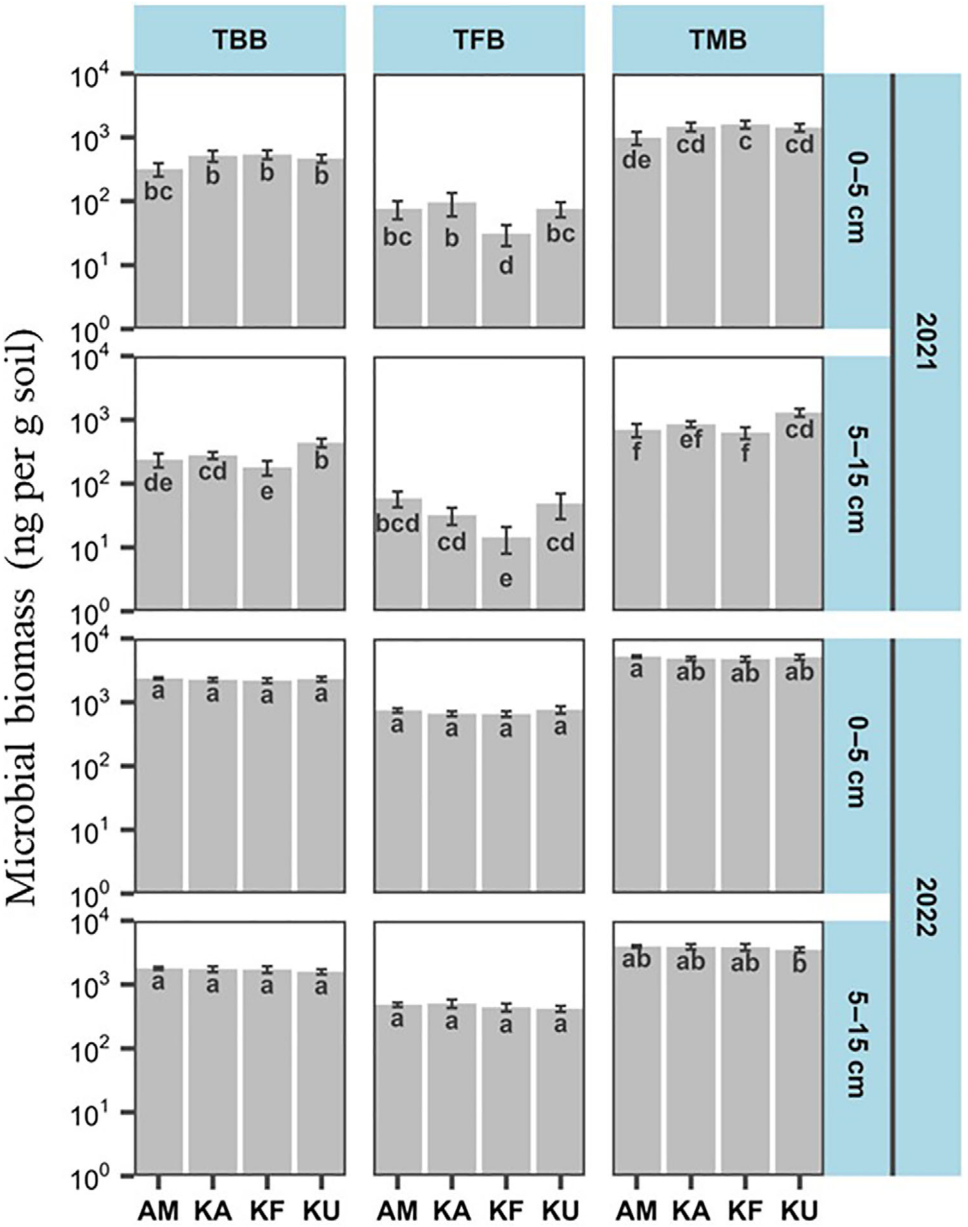

FIGURE 4 Post hoc test results showing mean comparisons of microbial biomass by treatments, depth, and year. Bars (mean ± SE) sharing the same letter do not differ ($p > 0.05$). AM, alfalfa monocrop; KA, Kernza and alfalfa intercrop; KF, Kernza fertilized; KU, Kernza unfertilized; TBB, total bacterial biomass; TFB, total fungi biomass; TMB, total microbial biomass.

crops' greater impact in microbial biomass compared to the monoculture.

3.4 | Principal components

Principal component analysis (Figure 5A) revealed that microbial community compositions and PLFA indices captured approximately 75.8% of the total variance due to the treatments, depth, and year with just PC1 (41.4%) and PC2 (34.4%). Fungi communities and saprophytes greatly contributed to the variance. Moreover, these variables were aligned in the same direction to one another, showing the presence of strong correlations between them. However, there was a clear clustering between the years (before and after intervention), showing a distinct effect of the management regime on microbial communities.

Constrained canonical correspondence analyses showed low effects of treatments, year, and depth on PLFA microbial communities (Figure 5B), although moderate effects were observed on community structure and stress ratios (Figure 5C). This suggests that the changes in those PLFA were due to other factors. This gives an insight about looking at some other specific environmental factors such as

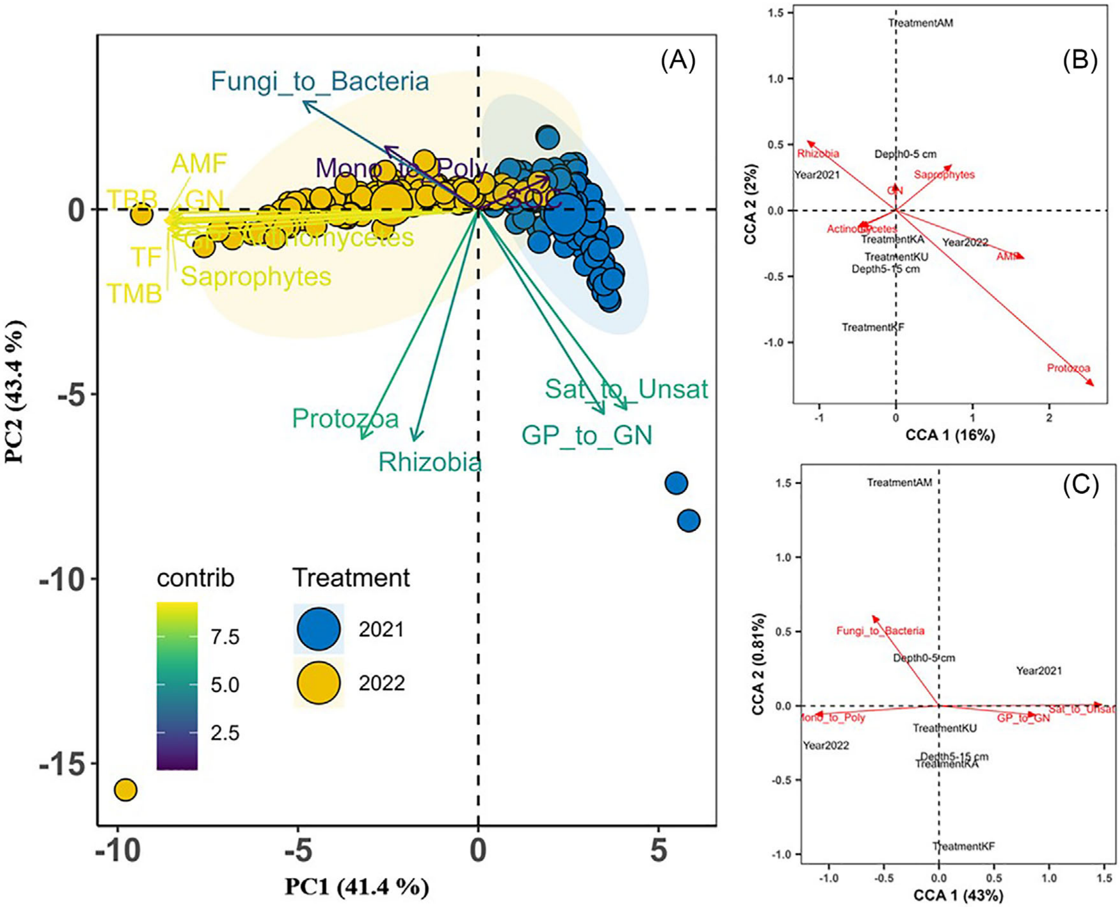

FIGURE 5 Principal component analysis (PCA) on microbial community indicators and stress ratios grouped by before and after intervention (years 2021 and 2022) (A), constrained canonical corresponding analysis (CCA) of microbial communities (B), and community and stress ratios (C). Variation contribution level of each variable in the PCA was denoted by color heatmap, increasing from blue to yellow. PCA plot shows the first (PC1) and second (PC2) principal components, whereas both CCA plots show first (CCA1) and second (CCA2) component values. AM, alfalfa monocrop; AMF, arbuscular mycorrhizal fungi; KA, Kernza and alfalfa intercrop; KF, Kernza fertilized; KU, Kernza unfertilized; GN, Gram-negative; GP, Gram-positive; GP/GN, Gram-positive/Gram-negative bacteria ratio; TF, total fungi biomass; TBB, total bacterial biomass; TMB, total microbial biomass; Mono/Poly, monosaccharides/polysaccharides ratio; Sat_to Unsat, saturated to unsaturated polysaccharide ratio; SOC, soil organic carbon.

moisture and temperature (Feng et al., 2003) or soil carbon fractions (e.g., labile and recalcitrant) when there is an interest to understand the soil factors that govern PLFA indicators. The arbuscular mycorrhizal fungi aligned in the direction of year 2022, indicating it has been benefiting from the perennial crops more than the other microbial groups. Interestingly, the GP/GN ratio corresponded with year 2021 and the opposite of year 2022, suggesting microbes were more stressed without than with perennial crops. Thus, the current study revealed that microbial stress condition may be related to lack of available carbon rather than drought and heat.

3.5 | Community and stress ratios

Like the individual parameters themselves, the ratios of PLFA microbial community biomass and stress ratios are robust soil health indicators (Norris et al., 2023). Results of two community ratios, namely, fungi/bacteria (Fun/Bac) and GP/GN, and a stress (Sa/Unsa FA) ratio, are presented in Figure 6. Like for the other PLFA profiles evaluated, effects of perennials cover on these ratios were observed in 2022 compared to 2021, before the plants were seeded. However, there was no difference among the treatments on these ratios.

The GP/GN ratio significantly decreased in both depths in year 2022 under every treatment, except AM. It ranged from 1.3–1.6 in the year 2022 compared to 1.3–6.4 in the year 2021 (Figure 2). It was interesting that the decline in the ratio was not because of the decrease in Gram-positive (GP) bacteria, but rather because of the greater rate of increase in Gram-negative (GN) bacteria, suggesting the latter were more favored with the change in the soil environment caused by those perennial crops. The GP bacteria are more resilient to changes in the environment compared to GN because of

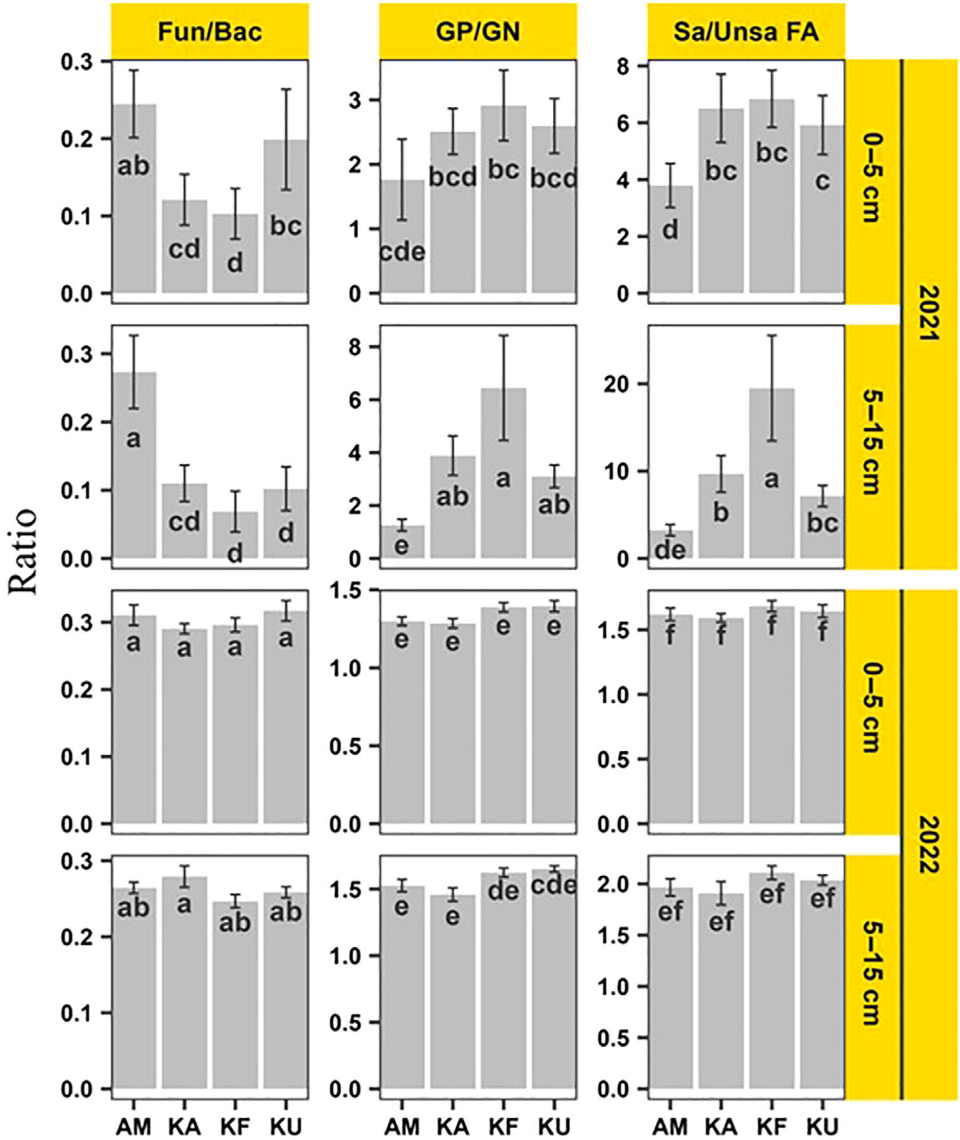

FIGURE 6 Post hoc test results showing mean comparisons of microbial community (Fun/Bac, fungi/bacteria; GP/GN, Gram-positive/Gram-negative) and stress (Sa/Unsa FA, saturated/unsaturated fatty acid) ratios by treatments, depth, and year. Bars (mean ± SE) sharing the same letter do not differ ($p > 0.05$). AM, alfalfa monocrop; KA, Kernza and alfalfa intercrop; KF, Kernza fertilized; KU, Kernza unfertilized.

their ability to produce spores. Thus, reduced GP/GN ratio in 2022 could indicate a decline in microbial stress due to the perennial crops, suggesting the significance of such crops in reclaiming soil health by improving availability of labile C in soils (Fanin et al., 2019). Also, several studies indicated that GN uses more plant-derived C source that are relatively labile, while GP uses C sources derived from SOC that are recalcitrant. They reported that the GP/GN ratio has significantly increased after removal of vegetation systems. Thus, the findings of current study imply that establishments of these perennial systems contributed to reduce the ratio of GP/GN. According to these results, the ratio of GP/GN may be considered a good indicator of soil health.

In contrast to GP/GN ratio, Fun/Bac ratio significantly increased in year 2022. A high Fun/Bac ratio is consistently desirable; the greater the value, the better is the soil health condition. A Fun/Bac ratio of >0.3 indicates an excellent health condition of a soil. It is also referred to as an excellent SOC content indicator (Frostegård & Bååth, 1996), but this is not always true, as also revealed by the current study. Microbial groups (e.g., fungi and bacteria) may not be favored by a management similarly (Chen et al., 2020; Srour et al., 2020). For instance, it is commonly understood that conventional farming like tillage and overgrazing may favor bacteria, whereas, contrary to this, no-till and cover crops may favor fungi more than bacteria. A greater Fun/Bac

TABLE 2 Partial least square path model (PLSM-PM) paths, path coefficients, and bootstrap ($n = 999$) validations for soil organic carbon (SOC) prediction and model validation at 95% confidence interval (CI).

	Variable	Original estimates			Bootstrap estimates and 95% CI		
		Estimate	*p*-value	Sig.	Estimate	perc.025	perc.975
0–5 cm							
	Gram-positive/Gram-negative	−0.041	0.714	ns	−0.04	−0.41	0.13
	Mono/poly FA ratio	−0.188	0.211	ns	−0.19	−0.60	0.03
	Saturated:unsaturated FA	0.538	0.001	**	0.54	**0.22**	**0.99**
	Fungi:bacteria	−0.175	0.033	*	−0.17	**−0.31**	**−0.02**
	R^2			0.24			0.27
5–15 cm							
	Gram-positive/Gram-negative ratio	0.157	0.116	ns	0.15	−0.05	0.31
	Mono/poly FA	0.615	0.024	*	0.64	**0.10**	**1.31**
	Saturated/unsaturated FA	−0.729	0.009	*	−0.77	**−1.43**	**−0.22**
	Fungi:bacteria	0.139	0.110	ns	0.13	−0.02	0.28
	R^2			0.14			0.16

Note: The bold CI ranges are non-zero inclusive, and these values validate the signficance of the orginal estimates.
Abbreviations: FA, fatty acid; Mono/poly FA, monounsaturated/polyunsaturated fatty acids; ns, non-signficant ($p > 0.05$).
Signficance (Sig.) levels: '*', 0.05; '**', 0.01.

ratio is desired, although bacteria are still important in soil ecosystems, mainly due to the positive associations with the ecosystem services such as nutrient recycling and carbon sequestration (Bailey et al., 2002; Malik et al., 2016). The increase of Fun/Bac ratio from 2021 to 2022 in the current study was also related to the succession of mycorrhizal FB, which was improved by the establishment of perennial species at the site as compared to the corn–soybean rotation in the previous year.

The pattern of Sa/Unsa FA ratio was like that of GP/GN ratio, but for AM, a significant effect was found only in the uppermost soil layer, 0- to 5-cm depth. It decreased from a mean value of 3.8–19.5 in 2021 to 1.6–2.0 in 2022. This ratio is also a measure of microbial stress (Kaur et al., 2005). Bacteria maintain their optimal fluidity by changing their membrane during environmental shocks. An increase in unsaturated fatty acid, which is a decrease in the Sa/Unsa FA ratio, could thus mean that some soil microbiota groups were under stress condition in 2022, but the reasons were not well established within the scope of the current study. However, it could be linked to increased soil temperature or drop in precipitation, which was lower by about 61 mm compared to the average rainfall from 1901 to 2000 (Missouri Climate Center, 2022) from August through September in 2022 (Figure 2). Such seasonal changes in precipitation could negatively affect soil moisture (Zuo et al., 2023), which in turn could result in increased competition for water between microbes and perennial crops in the rhizosphere.

3.6 | PLS-PM coefficient estimates and model validation

The potential of PLFA variables in predicting SOC was evaluated using the PLS-PM model for depths 0–5 cm and 5–15 cm separately, and results are shown in Table 2. Separate PLS-PM were developed for the two depths because the SOC content pattern remarkably varied under these depths. PLS-PM was useful to identify some of the PLFA indices that predict SOC content, but the PLFA ratios were related to SOC differently in the two depths. The predictive power was greater in the upper 0–5 cm with $R^2 = 0.24$ compared to $R^2 = 0.16$ in the lower depth (Table 2). In the upper soil depth, Sa/Unsa FA and Fun/Bac ratios had positive ($\beta = 0.54$, $p = 0.001$) and negative ($\beta = -0.175$, $p = 0.033$) relationships with SOC, respectively; however, this pattern changed in the 5- to 15-cm depth, and mono/poly and Sa/Unsa FAs had positive ($\beta = 0.615$, $p = 0.024$) and negative ($\beta = -0.729$, $p = 0.009$) relationships, respectively, with SOC. The change in pattern was likely linked to the dynamics of SOC content, which was not similar in the two depths (Figure 2) for the two sampling periods.

The GP/GN ratio was not useful to predict SOC in the current study. However, a previous report showed that it can be an excellent indicator for C availability for microbes in soil ecosystems (Fanin et al., 2019), because it is believed that GN utilize labile plant-derived C while GP rely on recalcitrant organic matter. Our findings also appeared to support this claim, as there was a significant decline in the ratio in 2022, which was mainly because of a greater rate of GN bacterial biomass increment compared to that of GP, and the presence of Kernza and alfalfa may have supplied plant-derived labile C that favored GN (Chamberlain et al., 2022; Fanin et al., 2019; Malik et al., 2016).

4 | CONCLUSIONS

Perennial crops could have several benefits that are linked to their functional roles with their deep roots and the management therein. However, there is lack of evidence on short-term effects of perennial grains on soil microbial communities and SOC. The current study was aimed at understanding short-term impacts of shifting lands to perennial cropping systems on microbial communities and structure. We hypothesized a shift from annual crop to perennial crop land use shifts microbial communities shortly after 1 year of the conversion. Perennials positively impacted microbial communities, although microbial structure has changed based on the conducive environment created with the perennial crops. Increased Fun/Bac ratio and decreased GP/GN bacterial ratio after 1 year with the perennials indicated reduced stress in microbial communities because of improvement in the soil environment, such as nutrient availability, moisture content, and temperature stability. Microbial parameters considered in the current study explained 80% of the total variance. Thus, these parameters were effective to understand the impacts of the perennials on soil biological health indicators. Microbial communities and Fun/Bac ratio greatly increased after 1 year with the perennial plants, irrespective of the treatment types, while GP/GN significantly decreased. Significant increments in Fun/Bac ratio and decline in GP/GN ratio, particularly, indicate improvements in the soil health. The overall study results show that both alfalfa and Kernza had positive impacts on PLFA microbial communities, even in short term, although relative benefits among the treatments were not observed. This suggests the importance of long-term interventions to realize relative benefits.

AUTHOR CONTRIBUTIONS

Ranjith P. Udawatta: Conceptualization; data curation; investigation; methodology; project administration; resources; supervision; validation; writing—review and editing. **Biyensa Gurmessa**: Formal analysis; validation; visualization; writing—original draft; writing—review and editing. **Miguel Salceda Gonzalez**: Data curation; methodology; validation. **Sidath S. Mendis**: Data curation; methodology; visualization; writing—review and editing. **Sarah T. Lovell**: Funding acquisition; methodology; project administration; supervision; validation.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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