



Article The Effect of Alternative Dryland Crops on Soil Microbial Communities

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Abstract: The composition of a soil microbial community that is associated with novel rotation crops could contribute to an increased yield of subsequent crops and is an important factor influencing the composition of the rhizosphere microbiome. However, the effect of alternative dryland crops on soil microbial community composition is not clear in the northern Great Plains (NGP). The objective of this study, therefore, was to evaluate the effects of the oilseed crops Ethiopian mustard (*Brassica carinata* A.) or camelina (*Camelina sativa* L.) or a 10-species forage/cover crop (CC) mix and fallow on soil biological health. Phospholipid fatty acid (PLFA) analysis was used to characterize the microbial community structure. The results showed that the total bacterial PLFA proportion was significantly higher under a CC mix compared to camelina and fallow. The fungal-to-bacterial ratio was significantly higher in CCs (0.130) and carinata (0.113) compared to fallow (0.088). Fungi are often considered a good indicator of soil health, while bacteria are crucial in soil functions. The changes in specific microbial communities due to crop-related alterations might play a key role in the yield of subsequent crops. This study provides valuable insights into the effect of oilseeds, CCs, and fallow on microbial communities.

Keywords: camelina; carinata; cover crop; crop rotation; PLFA

1. Introduction

Diversified crop rotations can affect soil health and resilience by increasing crop yields compared to monocropping and crop–fallow systems [1,2]. Studies have shown an increase in crop yields by including pulses and oilseed crops compared to monocropping and crop–fallow systems [3,4]. Industrial grade cool-season oilseed feedstocks such as carinata (*Brassica carinata*) and camelina (*Camelina sativa*) have shown production potential in semi-arid environments [5]. More than canola, camelina has a better tolerance to cold, heat, and drought and is less susceptible to diseases and pests [6]. However, among oilseed crops, Taylor et al. [7] observed a higher average yield in carinata than canola and camelina, which suggests a higher economic opportunity in carinata [7]. Camelina and carinata, both belonging to the Brassicaceae family, are of importance, as exudates of *Brassica* root contain glucosinolates (GSL), which hydrolyze to produce isothiocyanates (ITC) due to their allelopathic properties and are potent biofumigants [8,9]. In addition, cover crops (CCs) can add soil organic matter, improve soil fertility and increase soil microbial communities [10], improve soil structure [11], enhance N cycling [12], and help suppress some pests [13].

Despite their potential promise, there has been little research on the soil microbiology that is associated with these potential fallow replacement crops in the NGP region. Soil microorganisms are an integral part of many processes in the agroecosystem and play important roles in maintaining soil functions including decomposition, nutrient cycling, bioremediation, organic matter stabilization, and aggregate formation [14]. Shifts in the activity and composition of microbial communities can alter these processes due to



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Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). crop-related changes, environmental conditions such as soil moisture [15], or agricultural practices such as tillage, soil amendments [16], and cover cropping [17]. Thus, understanding how they respond to crop rotation, climate, tillage, soil type, etc., that influence the microbial community [18] can be indicative for the assessment of soil health for crop production.

A study by Bennett et al. [19] reported reduced soil fungal and bacterial communities with oilseed rape. Moreover, important microbial processes such as symbiotic associations between plants and microbes, nitrogen fixation, and nitrification could be disrupted with an exposure to the hydrolysis products of GSLs [20]. On the other hand, a study by Muhammad et al. [17] showed that fungi responded more than bacteria to CCs. Another study showed similar results where CC residues increased fungal populations, as soil fungi are more sensitive to CCs than bacteria [21]. Similarly, CCs enhanced arbuscular mycorrhizal fungal (AMF) growth and improved soil fertility and crop yield [22,23].

While a few studies have examined soil biology associated with canola and camelina [24–26] in the Pacific Northwest of the United States, to our knowledge, there are no reports on soil microbial communities in carinata, camelina, or multispecies CCs that dominate in the highly variable rainfall environments that are typical of the NGP region. The objectives of this research were to (1) determine the differences and similarities in the rhizosphere microbial communities from cropping alternatives to fallow in a wheat–fallow cropping system and to (2) identify the effect on soil biological health of non-food bioenergy feedstocks and 10-species CCs in the dryland cropping systems of the NGP. This study was conducted from 2014 to 2020 to evaluate the effects of three crops planted in place of fallow on soil microorganisms in durum. This study is a part of integrative research designed to assess the potential of diversifying the traditional wheat–fallow cropping system, which is typical of dryland production systems, by replacing the fallow phase of the rotation with a suitable crop. We hypothesized that the soil microbial communities in oilseed crops would be differentiated from one another, as well as from 10-species CC/forage mix and fallow.

2. Materials and Methods

2.1. Study Site

The study was conducted from 2014 to 2020 at the Northern Plains agricultural research laboratory's dryland experimental farm $(48^{\circ}33' \text{ N}, 104^{\circ}50' \text{ W}, altitude 589 \text{ m})$, situated about 11 km north of Culbertson, Montana [27]. The soil was Dooley sandy loam (fine-loamy, mixed, superactive, frigid Typic Argiustolls). In the Ap horizon (0–15 cm), the quantity of sand, silt, and clay were around 62%, 22%, and 16%, respectively. The pH of the soil sample at the 0–20 cm depth prior to the start of this field study was 7.2, and the organic matter content was 1.7% [27,28]. The cropping system at this site was spring wheat–summer fallow or continuous spring wheat for over 30 years.

The study consisted of four treatments: durum wheat (*Triticum durum* D.) planted in a 2-year rotation either with fallow or with one of three potential fallow replacement crops. The three replacement crops trialed were camelina (*Camelina sativa* L.), carinata (*Brassica carinata* A.), and a 10-species cover crop mix. The CC mix included a functionally diverse mix of warm and cool season plants, broadleafs, and grasses including 19% forage pea (*Pisum sativum* L. spp. *Sativum*) and cowpea (*Vigna unguiculata* (L.) Walp.); 13% forage sorghum (*Sorghum bicolor* (L.) Moench) and buckwheat (*Fagopyrum esculentum* Moench); 12% radish (*Raphanus sativus* L.), flax (*Linum usitatissimum* L.), and german millet (*Setaria italica* L.); and 6% lentil (*Lens culinaris* Medik.), turnip (*Brassica campestris* L.), and winter canola (*Brassica napus* L.). To maximize ecosystem services, the diversified CC mix was planted, including pollinator-friendly species. Highly diversified cover crop mix was planted due to a highly variable climate in the region that typically favors different species in terms of productivity between different years.

In late April to early May, planting for all crops in the study took place using a custom-built no-till research drill with double-shoot Barton single-disk openers on 20.3 cm

row spacing. Durum "Grenora" was planted at 67 kg ha⁻¹. *Camelina* "Suneson", *carinata* "A110", and the cover crop mix were planted at 9.0, 7.3, and 26.9 kg ha⁻¹, respectively. Oilseeds were harvested with a Kincaid 8XP research combine (manufactured in Haven, KS, USA) in July–August. The cover crop mix was swathed for forage at pea bloom in mid-July. The cover crop was allowed to regrow unharvested and terminated each year with a killing frost following forage harvest.

All plots received 56 kg ha⁻¹ monoammonium phosphate and 45 kg ha⁻¹ potassium chloride, and oilseed crops plots received 112 kg ha⁻¹ ammonium sulfate and urea for 1680 kg ha⁻¹ yield goal banded at planting [27]. At planting, fertilizer was banded 5 cm below and 5 cm to the side of the seed. Pre-plant and post-harvest applications of glyphosate and 2, 4-D herbicides were used for weed control. Durum received an in-crop application of bromoxynil and fenoxaprop herbicides at labeled rates, and oilseed crops received ethylfluralin.

Oilseeds, cover crop mix, and fallow always followed a previous durum crop. Research plots were arranged in randomized complete block design with three replications. We presented 18 plots in total from three different rotations, where each crop phase of the rotation was included. Each plot measured 6 m wide \times 15 m long. The chemical characteristics of the field plots measured in 2020 are summarized in Table 1, where concentration of nitrate was significantly higher in camelina compared with fallow, and concentration of iron was significantly higher in fallow compared with carinata, whereas CEC was significantly higher in fallow and oilseed crops compared with CCs.

Soil Factor CC Camelina Carinata Fallow $EC (ds m^{-1}) *$ 0.37 0.44 0.31 0.43 SOM $(g kg^{-1})$ 1.93 2.132.03 1.96 37.20 ab † NO_3^- (mg kg⁻ 48.70 a 47.06 ab 34.60 b K (mg kg⁻ 270.66 305.00 298.00 280.66 $S (mg kg^{-1})$ 10.23 15.00 13.40 12.16 $Zn (mg kg^{-1})$ 0.89 0.91 0.80 0.95 Fe (mg kg^{-1}) 52.10 ab 53.71 ab 46.86 b 63.60 a $Mn (mg kg^{-1})$ 66.66 69.93 63.76 81.33 Cu (mg kg^{-1}) 1.29 1.45 1.22 1.68 Ca (mg kg^{-1}) 757.33 867.33 1040.00 1140.00 $Mg (mg kg^{-1})$ 269.66 287.00 325.00 272.00 Na (mg kg^{-1}) 11.33 12.66 12.66 12.00 CEC (Cmol_ckg⁻¹) 11.40 b 12.76 a 12.73 a 12.46 a HBS% 35.66 34.33 27.33 45.33 K BS % 6.00 6.33 5.66 6.00 Ca BS % 40.00 30.33 37.66 44.66 Mg BS % 20.00 18.66 21.33 18.33 M-3P(mg/kg)54.00 52.66 46.0049.66

Table 1. Soil chemical properties for CC, camelina, carinata, and fallow.

⁺ Means followed by different lowercase letter within a row are significantly different at $p \le 0.05$. * EC, electrical conductivity; SOM, soil organic matter, NO₃⁻, Nitrate; K, potassium; S, Sulfur; Zn, Zinc; Fe, Iron; Mn, Manganese; Cu, copper; Ca, Calcium; Mg, Magnesium; Na, Sodium; CEC, Cation Exchange Capacity; H, Hydrogen; P, Phosphorus.

2.2. Soil Sampling

Soil sample collection occurred each year in spring (May–June) from 2015 to 2020. Each sample for microbial community composition and soil chemical properties consisted of five composited cores to a depth of 20 cm using a 3 cm diameter probe. Samples were placed in sealed plastic bags, stored on dry ice immediately after collection, and then placed in a -20 °C freezer in the laboratory until analyzed.

2.3. Phospholipid Fatty Acid, Neutral Lipid Fatty Acid, and Soil Chemical Analyses

Phospholipid fatty acid (PLFA) analysis was used to measure microbial groups following methods reported previously [29]. Briefly, lipids were separated by solid-phase extraction (SPE). The PLFA and neutral lipid fatty acid (NLFA) fractions were converted to produce fatty acid methyl esters and were qualitatively and quantitatively analyzed by gas chromatography using a flame ionization detector [29]. Fatty acids were identified using peak naming software and a library of standards (PLFAD1) from MIDI Inc. (Newark, DE, USA). Fatty acids were summed into biomarker groups: Gram-positive bacteria were quantified by the presence of iso- and anteiso-saturated branched fatty acids; Gram-negative bacteria with monounsaturated fatty acids and cyclopropyl 17:0 and 19:0; eukaryotes with polyunsaturated fatty acids; and actinomycetes with 10-methyl fatty acids [30,31]. Fungi were identified and quantified with 18:2 ω 6c and arbuscular mycorrhizal fungi (AMF) with 16:1 ω 5c [32]. By dividing the sum of the fungal fatty acid markers by the sum of the bacterial fatty acid markers, a fungal-to-bacterial (F/B) ratio can be determined [32]. The PLFA and NLFA 16:1 ω 5c has been shown to be a good indicator for AMF active biomass and stored energy, respectively [33].

Soil chemical analyses were performed at Ward Laboratories, Kearney, NE, USA. Briefly, organic matter concentration was measured using the loss on ignition method; NO₃–N concentration using an auto-analyzer after extracting the soil with 2 M KCl solution; K, Ca, Mg, and Na concentrations using the atomic absorption spectrometry after extracting the soil with NH₄OAC; and P concentration using a colorimeter after extracting the soil with the Mehlich-3 solution. The cation exchange capacity (CEC) was calculated by summing the concentration of Ca, Mg, K, and Na. Base saturation for each cation was calculated by dividing the concentration of each cation by the sum of all basic cations. Soil chemical properties were measured only in 2020.

2.4. Statistical Data Analyses

Two-way analysis of variance was used to analyze soil chemical data. Also, analyses of variance using Proc GLIMMIX [34] for a split-plot design was conducted on the proportion of each biomarker. Crop was the whole-plot factor, year was the subplot factor, and their interaction was considered the fixed effect. Means separation tests were conducted using Tukey's Honestly Significant Difference (HSD). Fatty acids were summed into biomarker groups [29]. Canonical multivariate analysis was used to compare soil microbial communities in different years. Multivariate analysis on the relative area of each biomarker was used to identify the linear combination of variables (referred to as canonical variates) that best separated soil microbial community structure at different previous crops. The canonical variates were graphed to summarize group differences [35]. All statistical analyses were performed at the p < 0.05 significance level. Because of the establishment year of crops in 2014 and the hail damage of crops in 2018, data for these two years were not included in statistical analysis.

3. Results

3.1. Precipitation

The total precipitation during the 2015, 2017, and 2020 cropping seasons was lower than the 30-year average, marked by an unusually dry period in April 2015, 2017, and 2020 and August and September in 2020 (Table 2). The total precipitation during the 2018 growing season was slightly higher than the 30-year average. However, the 2016 and 2019 cropping seasons were characterized by an unusual abundance of precipitation, especially during the early growing season (April and May) in 2016 and June, August, and September in 2019.

| | Precipitation, mm | | | | | | | | | |
|--------------|-------------------|-------|-------|-------|-------|-------|--------------------|--|--|--|
| Month – | 2015 | 2016 | 2017 | 2018 | 2019 | 2020 | 30-yr ⁺ | | | |
| April | 5.5 | 42.2 | 2.75 | 31.5 | 27.5 | 3.25 | 24.1 | | | |
| May | 40.5 | 83.5 | 19.5 | 46.2 | 29.5 | 52 | 56.7 | | | |
| June | 81.0 | 50.5 | 22.7 | 67.7 | 83.5 | 33.7 | 72.1 | | | |
| July | 35.5 | 69.0 | 24.7 | 84.7 | 30.7 | 70.2 | 67.5 | | | |
| August | 54.0 | 10.2 | 26.5 | 20.2 | 68.2 | 4.75 | 35.5 | | | |
| September | 38.2 | 95.5 | 84.5 | 45.0 | 156.7 | 2.5 | 33.2 | | | |
| Season total | 254.7 | 351.0 | 180.7 | 295.5 | 396.2 | 166.5 | 289.1 | | | |
| Yearly total | 329.5 | 443.7 | 247.5 | 383.2 | 464.2 | 213.2 | 356.8 | | | |
| | | | | | | | | | | |

Table 2. Monthly precipitation from April to September during 2015–2020 growing seasons at the experimental site near Culbertson, MT.

⁺ Thirty-year average (1991–2020) from Western Regional Climate Center for Culbertson, MT, located 11 km south of the research site.

3.2. Biomarker Values and Soil Microbial Community Structure for CCs, Camelina, Carinata, and Fallow

The years varied greatly for the arbuscular mycorrhizal fungi (AMF) phospholipid fatty acid (PLFA) proportion, where the higher AMF proportion across crops occurred during the 2020 season compared with the 2017 and 2019 seasons (Table 3). The saprophytic fungal PLFA proportion varied between crops and years, but the crop \times year interaction was not significant. The saprophytic fungal proportion was significantly higher in CCs compared to fallow and a higher saprophytic fungal proportion across crops occurred in 2019 compared to 2017 and 2020.

Table 3. Arbuscular mycorrhizal fungal, Gram-negative (Gm–), eukaryotic, fungal, Gram-positive (Gm+), actinomycetes, total fungal and bacterial PLFA, fungi/bacteria, Gm+/Gm– PLFA, and AMF NLFA proportions in two Brassicaceae oilseeds, a cover crop mix (CC mix), and fallow near Culbertson, MT, 2017–2020.

| Crop (C) | AMF PLFA | Gram — Bacteria | Eukary | Fungal | Gram + Bacteria | Actino | Total Fungal | Total Bacte- rial | F/B | Gm +/Gm– | AMF NLFA |
|--------------|-------------|--------------------|---------|----------------------|--------------------|----------|-----------------|-------------------------|----------|-------------|-------------|
| CC | 0.041 | 0.249 | 0.246 | 0.070 a [†] | 0.450 b | 0.155 b | 0.111 a | 0.863 b | 0.130 a | 1.836 | 0.044 |
| Camelina | 0.038 | 0.232 | 0.017 | 0.051 ab | 0.480 a | 0.177 a | 0.090 b | 0.893 a | 0.101 ab | 2.100 | 0.044 |
| Carinata | 0.038 | 0.228 | 0.022 | 0.060 ab | 0.472 ab | 0.168 ab | 0.099 ab | 0.878 b | 0.113 a | 2.083 | 0.033 |
| Fallow | 0.036 | 0.232 | 0.022 | 0.043 b | 0.486 a | 0.174 a | 0.079 b | 0.898 a | 0.088 b | 2.151 | 0.039 |
| Year | | | | | | | | | | | |
| 2017 | 0.035 b | 0.231 | 0.020 b | 0.042 b | 0.486 a | 0.172 | 0.077 b | 0.902 a | 0.086 b | 2.116 | 0.038 |
| 2019 | 0.035 b | 0.233 | 0.014 b | 0.076 a | 0.469 ab | 0.165 | 0.112 a | 0.873 b | 0.129 a | 2.061 | 0.043 |
| 2020 | 0.045 a | 0.240 | 0.029 a | 0.050 b | 0.462 b | 0.170 | 0.095 b | 0.874 b | 0.109 a | 1.950 | 0.040 |
| Signifi | icance | | | | | | | p v | alue | | |
| C | ns ‡ | ns | ns | * | * | * | * | * | * | ns | ns |
| Y | * | ns | * | * | * | ns | * | * | * | ns | ns |
| $C \times Y$ | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | * |

* Significance $p \le 0.001$; [†] means followed by different lowercase letter within a column are significantly different at $p \le 0.05$; [‡] not significant.

The Gram-positive bacterial PLFA proportion varied between crops and years, but the crop \times year interaction was not significant. The Gram-positive proportion was significantly higher in fallow and camelina compared to CCs and was significantly higher in proportion in 2017 compared to 2020. Whereas the actinomycetes proportion varied between crops, the year and crop \times year interaction was not significant. The actinomycetes proportion was significantly higher in fallow and camelina compared to the CC mix.

The total bacterial and total fungal PLFA proportion of oilseed and CC crops and fallow varied by crop and year, but the crop \times year interaction was not significant (Table 3). The total fungal proportion varied between years and crops. The total fungal proportion was significantly higher in the CC mix compared to oilseed crops and fallow. The years

varied greatly for total fungal proportion, where the higher fungal proportion across crops occurred during the wet 2019 season. Across years, fallow and camelina consistently had the highest total bacterial proportion, while CCs had the lowest and carinata was intermediate. The years varied greatly for total bacterial proportion, with the highest proportion across crops occurring during the dry season. The fungal/bacterial ratio varied for crop and year. Cover crops and carinata had a similar F/B ratio, and fallow accumulated a smaller F/B ratio, likely due to poor biomass. Across years, the F/B ratio was higher in wetter years (2019) compared to drier years (2017 and 2020).

The AMF NLFA proportion was not consistent for crops across years, resulting in a significant crop \times year interaction (Table 3). In 2017, CCs had the highest AMF NLFA proportion, while carinata had the smlest proportion (Table 4). Conversely, in 2019, fallow and camelina had the highest AMF proportion, and CCs and carinata had the smallest.

Table 4. Interaction between crop and year for AMF NLFA proportions for two Brassicaceae oilseeds and a cover crop mix (CC mix) near Culbertson, MT, 2017–2020.

| Crop | 2017 | 2019 | 2020 |
|----------|----------------------|---------|-------|
| | AMF | NLFA | |
| CC | 0.053 a [†] | 0.029 b | 0.054 |
| Camelina | 0.033 ab | 0.064 a | 0.035 |
| Carinata | 0.028 b | 0.023 b | 0.047 |
| Fallow | 0.036 ab | 0.054 a | 0.028 |

⁺ Means followed by different lowercase letter within a column are significantly different at $p \le 0.05$. Standard errors are in parentheses.

Canonical multivariate analysis indicates that differences in the soil microbial communities among different rotation crops were significant (Figure 1). The microbial communities in CCs and oilseeds were the most distinct in the crop years 2017, 2019, and 2020. The soil microbial communities share similarities between CCs and carinata in 2017 and CC and camelina in 2019, as shown by the closer proximity of biomarker clusters to one another. There was an increasing trend of a variation pattern among the microbial communities over the four-year period, regardless of seasonal fluctuations. The biomarker clusters of carinata in 2019 and CCs in 2020 were separated distinctly from the other soil microbial communities (Figure 1).

The ability of discriminant functions to differentiate among different rotation crops on the basis of amounts and types of PLFAs were found to be significant (Table 5). In 2017, canonical variate (CV) 1 discriminated CCs and carinata versus camelina and fallow, and CV 2 discerned CC from carinata and camelina from fallow. Samples collected from cover crops contained relatively higher AMF, fungal, actinomycetes, and Gram-negative and Gram-positive bacterial PLFAs. In 2019, CV 1 separated CCs and fallow versus carinata, and CV 2 differentiated CCs versus fallow. Carinata contained higher fungal communities. In 2020, CV 1 discriminated CC from carinata, and CV 2 separated samples collected from CCs and oilseed crops from fallow. Soils under CCs were higher in all PLFA biomarkers (Table 5).

Table 5. Structure matrix (pooled with canonical structure) and function at Group Centroid for CC, camelina, carinata, and fallow for years 2017, 2019, and 2020.

| Demonstration | 2017 | | 2019 | | 2020 | |
|--------------------|-------|-------|-------|--------|-------|--------|
| rarameter | CV1 | CV2 | CV1 | CV2 | CV1 | CV2 |
| Structure loadings | | | | | | |
| AMF | 0.209 | 0.849 | 0.131 | 0.088 | 0.473 | -0.429 |
| Gram—bacteria | 0.281 | 0.823 | 0.102 | 0.069 | 0.439 | -0.312 |
| Eukaryotes | 0.016 | 0.661 | 0.036 | 0.084 | 0.382 | -0.118 |
| Fungi | 0.133 | 0.576 | 0.249 | -0.292 | 0.397 | -0.217 |

| Tab | le 5. | Cont. |
|-----|-------|-------|
|-----|-------|-------|

| D (| 20 | 2017 | | 2019 | | 2020 | |
|-----------------|--------|--------|--------|--------|--------|--------|--|
| Parameter | CV1 | CV2 | CV1 | CV2 | CV1 | CV2 | |
| Gram + bacteria | 0.322 | 0.682 | 0.180 | 0.093 | 0.371 | -0.258 | |
| Actinomycetes | 0.272 | 0.746 | 0.211 | 0.149 | 0.344 | -0.269 | |
| Group centroids | | | | | | | |
| CC | -1.615 | 0.844 | -1.788 | -1.378 | 2.751 | -0.314 | |
| Camelina | 1.307 | -0.300 | -0.506 | 0.634 | -0.998 | -0.187 | |
| Carinata | -1.433 | -0.851 | 3.415 | -0.289 | -1.375 | -0.868 | |
| Fallow | 1.740 | 0.308 | -1.120 | 1.032 | -0.376 | 1.371 | |



Figure 1. Canonical multivariate analysis of variance of phospholipid fatty acid biomarkers in two oilseeds (carinata and camelina), cover crop mix, and fallow. Vectors represent standardized canonical coefficients and indicate the relative contribution of each biomarker group to each canonical variate.

4. Discussion

Soil moisture content is an important factor affecting soil's biological properties [36], and the decrease in moisture content may lead to a reduction in microbial biomass [37]. An increased soil water availability due to timely rainfall during the growing season in

2019 (Table 2) may have stimulated the growth of fungal communities compared to a dry year (2017). It may be possible that an increased soil water availability due to enhanced precipitation also increased the crop yield [38–40], which promoted nutrient substrate availability, thereby further enhancing microbial growth. Exceptions, however, occurred for bacterial communities, mainly Gram-positive bacteria, which were greater in 2020 when precipitation was lower (33.7 mm precipitation during June). It is likely that both Gram-positive bacteria and actinomycetes appear to be more resistant to drought than Gram-negative bacteria because of their thicker peptidoglycan cell wall layer [41]. The greater F/B ratio in 2019 and 2020 compared to 2017 indicates that soil moisture enhanced the substrate accessibility for efficient breakdown of complex and simple compounds by fungi and bacteria, respectively [42].

Changes in microbial abundance and community structure can directly and indirectly, be affected by crop species, rotation sequence, and cropping intensity [43,44]. Studies have shown the increased effects of cover cropping on soil microbial abundance, microbial enzymatic activities, and soil health [45]. The greater fungal population in cover crops may be due to an increased C and N input from CC residues [17], which stimulates microbial growth. Studies also found that fungi responded to cover crops more than bacteria, and fungal organisms are associated with the presence of plants and/or litter [46,47]. Considering the importance of soil microbes in maintaining soil health, our findings suggest that 10-species cover crops can enhance total fungal proportions compared to fallow and camelina.

In contrast, the greater total bacterial proportion in fallow and camelina compared to carinata and CCs indicates that bacteria tend to dominate in systems with fewer organic inputs or residues, as observed in another study [7]. Moreover, Hansen et al. [26] observed a decline in soil fungal abundance in camelina and concluded that this may be due to the exposure to residues and root exudates of camelina. The increased Gram-positive bacteria and actinomycetes in camelina and fallow compared with CCs were probably a result of lower nutrient substrate availability. Several researchers have reported that Gram-positive bacteria including actinomycetes tend to be associated with a lower availability of organic C substrates [48], in contrast to Gram-negative bacteria and fungi that strongly depend on input of fresh organic materials [49].

The greater F/B ratio for CCs and carinata compared with fallow indicates that increased root biomass and soil organic C enhanced the growth of fungi more than bacteria under both CC and carinata compared with fallow. Studies have shown that carinata has a greater yield potential and higher cold tolerance compared to other oilseeds like canola and camelina and provide ecosystem services that are similar to other CCs [50]. It has been shown that the F/B ratio is linked to higher soil organic C [51] and that soil fungi may be a more reliable indicator of increased soil C storage compared with bacteria [52].

Our findings suggest that oilseeds, cover crops, and fallow can have significant effects on soil microbial communities. A decline in viable microbial communities due to the practice of summer fallow could interrupt key microbial processes that are related to nutrient and water acquisition and essential for optimal crop production. As fungal communities are recognized as a good indicator of soil C storage, even a small increase in fungal abundance could be significant for semi-arid regions with low SOC levels. The goal is to decrease the practice of summer fallow and increase the use of diversified crop rotation to increase organic inputs to help soil support and enhance beneficial microbial communities that are already present in the soil.

5. Conclusions

Adopting a novel crop diversification method is an important management tool for enhancing soil microbial abundance in agroecosystems. Root exudates induce changes in the microbial community composition that affect the performance of subsequent crops. Results from a field study showed that cool-season oilseed crops influenced the soil microbial communities and differentiated them from the community associated with a CC mix. The total bacterial proportion was significantly higher in camelina and fallow, and the total fungal proportion was significantly higher under a CC mix. An increase in fungal communities under cover crops could be profitable in semi-arid regions, as fungi contribute more C storage compared to bacteria. Changes in the abundance of specific rhizosphere microbial groups due to oilseed and cover crops may play a key role in the crop yield and overall soil health. Our findings provide information for the scientific community and dryland farmers on the effect of camelina, carinata, and a CC mix on soil microbial communities as the land area planted with CCs and oilseed crops are expected to increase in future years.

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