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Continuous Monoculture of Alfalfa and Annual Crops Influence Soil Organic Matter and Microbial Communities in the Rainfed Loess Plateau of China

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Abstract: Cropping systems are structured to maximize crop yields and increase sustainability in agricultural production. A field study was conducted to investigate different long-term cropping systems on soil organic matter and microbial communities. The cropping systems studied were: (i) a 14-year continuous alfalfa (*Medicago sativa* L.) (CA), (ii) a 9-year alfalfa removed and rotated with 4–5 years continuous annual crops (spring wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), potato (*Solanum tuberosum* L.), and millet (*Panicum miliaceum* L.)), and (iii) a 5-year field fallow after alfalfa. Results showed that continued annual crops decreased total organic C and labile organic C by 10 to 20% and 17 to 34% in the topsoil (0–30 cm), and by 15 to 35% and 20 to 46% in the subsoil (30–60 cm), respectively, compared with CA. Similar trends were found in soil total N concentration, which decreased by 7 to 20% in the topsoil. Highest microbial biomass C was found in CA. Shannon-Wiener diversity and substrate richness of soil microbes measured by Biolog EcoPlates was significantly affected by cropping system with CA exhibiting a higher degree of soil microbial functional diversity in the topsoil, while the lowest values were found in the alfalfa-potato system. The higher soil organic matter content and functional diversity of soil microbe in CA indicates that soil nutrition and microbial activity did not limit alfalfa development and growth in the dryland area. The lower microbial activity and functional diversity observed in the potato field indicates the importance of crop selection in cropping systems.

Keywords: continuous monoculture; Biolog EcoPlates; rainfed cropping systems; microbial biomass C and N; functional diversity

1. Introduction

In the northwestern Loess Plateau of China, cropping systems are dominated by continuous cereal crops. Extensive residue removal, tillage, and the cultivation of sloping land have contributed to severe soil erosion and water loss. Including legume crops in cropping systems can enhance residual soil water and N availability, and improve system productivity [1,2]. Alfalfa is a perennial legume, has a high ability for root nodulation with N₂-fixing *Rhizobia* and produces numerous fibrous roots which increase soil organic matter [3] and improve soil aggregate structure [4]. The inclusion of alfalfa in cereal-based cropping systems is a traditional practice in the northwestern Loess Plateau of China,

and alfalfa has been used as a major forage crop for livestock feed. Alfalfa is also used for restoring degraded soils and improving soil quality, and its area of production has been expanding annually on the Loess Plateau of China. However, long-term continuous alfalfa in rainfed environments depletes soil water, thus reducing productivity both of alfalfa and subsequent grain crops [5]. Fan et al. [6] found that soil water storage following a five-year-old stand of alfalfa was 200 mm less compared with the initial soil water. Luo et al. [7] reported that alfalfa yield began to decline after eight years, due to severe depletion of soil water storage in the Loess Plateau of China.

Alfalfa-annual grain crop rotations are conducive to improve the stability and sustainability of agricultural systems. Alfalfa rotated with annual crops can increase soil nitrogen use efficiency [8,9] and recharge water at 0–180 cm soil layer, where alfalfa was grown previously [10]. Numerous studies have investigated the effect of alfalfa on the soil water regime of farming systems with different annual patterns of rainfall [10–13], and on soil physicochemical properties [14–16], whereas relatively few researches have observed the characteristics of soil microbial communities in continuous alfalfa and alfalfa rotated with annual crops.

Soil microorganisms play an important role in soil physicochemical processing and contribute to improve soil quality. Soil living organisms influence various ecosystem processes, including decomposition of organic matter [17], recycling of nutrients and trace elements, modification of soil physical and chemical properties, and suppression of pests and diseases [18–20]. Crops play a major role in shaping soil microbial communities. The composition and content of root exudates differ with various crops, thus altering soil microbial growth and metabolism [21]. There is evidence that soil microbial biomass declines in fields under long-term continuous annual crops because of the combined effects of soil physicochemical properties, root exudates, and biological changes [22]. Niu et al. [23] reported that intensified rotations including pea (*Pisum sativum* L.) build up pea rhizosphere pathogens in cereal and pulse-based cropping systems. Wang et al. [24] also found that continuous soybean (*Glycine max* (L.) Merr.) reduces the total number of soil microbes, and this effect increases with years of continuous cropping; in contrast, soybean rotated with cereals is conducive to maintaining soil microbial diversity and activity.

The objective of this study was to evaluate the effects of continuous alfalfa (14 years), continuous annual crops and field fallow after alfalfa removed after 9 years on: (i) the characteristics of soil microbial community, and (ii) properties of soil organic matter. We hypothesized that (i) soil organic matter would be important factors structuring the soil microbial communities, and (ii) monoculture crops generated different microbial communities and their own carbon resource characteristics. To test the hypothesis, the soil total organic C, labile organic C, soil microbial community-level physiological profile (CLPP) and microbial biomass C and N was determined in the alfalfa experiment at the rainfed agricultural system of the Loess Plateau of China.

2. Materials and Methods

2.1. Site Description

The field experiment was conducted at the Dingxi Experimental Station of Gansu Agricultural University (35°28' N, 104°44' E, 1971 m a.s.l.), Gansu Province, northwest China. The site had a Huangmian soil, aligning with calcaric cambisols in the FAO soil map of the world. It is a sandy loam with low fertility, representing the major soil type under crop cultivation in the Loess Plateau. Averaged long-term annual rainfall is 391 mm, with about 54% of the rainfall occurring from July to September. Daily maximum temperatures can be up to 38 °C in July, while minimum temperatures can drop to −22 °C in January. The ≥ 10 °C annual accumulated temperature is 2239 °C, and radiation is 5929 MJ m^{−2} with 2470 h of sunshine per year.

2.2. Experimental Design and Treatment Description

The experiment was a completely randomized block design with six treatments. Alfalfa (cv. Longdong 3) field was established in 2003 with a seeding rate of 18 kg of pure live seed ha⁻¹. It was removed in 2012 and rotated with spring wheat (cv. Dingxi 42), maize (cv. Xianyu 335), potato (cv. Xindaping), and millet (cv. Longgu 11) crops. The plot size was 3-m × 7-m with three replicates totaling 18 plots. Treatment and experiment details are shown in Table 1.

Table 1. Details of treatments and their descriptions.

Treatment	Abbrev	Description
Continuous alfalfa	CA	Alfalfa established in 2003 and continuously cropped to 2016
Alfalfa-fallow	AF	Alfalfa established in 2003 and removed in March 2012, field continuously fallowed to 2016
Alfalfa-fallow-wheat	AFW	Alfalfa established in 2003 and removed in March 2012, field fallowed until spring wheat was sown in spring 2013, and spring wheat continuously cropped in 2014–2016
Alfalfa-fallow-maize	AFC	Alfalfa established in 2003 and removed in March 2012, field fallowed until maize was sown in May 2013, and maize continuously cropped in 2014–2016
Alfalfa-potato	AP	Alfalfa established in 2003 and removed in March 2012, potato was sown in May 2012 and continuously cropped in 2013–2016
Alfalfa-millet	AM	Alfalfa established in 2003 and removed in March 2012, millet was sown in May 2012 and continuously cropped in 2013–2016

For the annual crops, spring wheat, and millet were sown by a locally designed traditional seeder (6 rows in 1.2 m width), with seeding rate of 187.5 and 15 kg ha⁻¹, respectively. Maize was sown with alternate wide (0.7 m) and narrow (0.4 m) ridges at a density of 52,500 plants ha⁻¹, as described by Lamptey et al. [25] Potato was sown using a plant spacing of 0.30 m to achieve a density of 52,500 plants ha⁻¹. All the annual crops were planted with conventional tillage, where the field was annually rotary ploughed twice: once in fall after crop harvest and the other in spring when sown, while crop residue was removed manually from the plots after harvest. All annual crops were fertilized with phosphorus (P₂O₅) at 105 kg ha⁻¹ and with N at 105 kg ha⁻¹, except maize plots, which received 105 kg P₂O₅ and 200 kg N ha⁻¹. For each year, the millet, potato, and maize were always planted at the first week of May and harvested in the first week of October, whereas spring wheat was planted during the last week of March and harvested the first week of August. Alfalfa was cut twice for each year in June and October, respectively.

2.3. Soil Sampling

Sampling was conducted at the first cut of alfalfa peak flowering stage in June 2016. Soil samples were collected from 0–30 and 30–60 cm soil depth during crop growing season. Five cores (25 mm in diameter) were taken and bulked into one sample for each plot. After the samples were thoroughly mixed, it was divided into two parts. One portion of soil sample was dried and sieved to 2 mm size for soil organic carbon compositions measurement, and the other portion was stored at 4 °C for soil microbial biomass carbon (MBC)/nitrogen (MBN) and microbial CLPP evaluation.

2.4. Soil C and N

Soil total organic C (TOC) and nitrogen (TN) were measured by dry combustion method using an Elementar CN-analyzer (vario MACRO cube, Germany). Labile organic carbon (LOC) was determined by potassium permanganate oxidation [26] with potassium permanganate concentrations of 333 mmol/L. Soil microbial biomass C/N was evaluated by fumigation extraction technique with $k_{EC} = 2.64$ [27] and $k_{EN} = 1.85$ [28].

2.5. Microbial Community-Level Physiological Profiling

Soil microbial community-level physiological profiling was evaluated by the classification and characterization of heterotrophic microbial communities, based on the sole carbon source utilization patterns. Biolog EcoPlates (EcoPlate™, Biolog Inc., Hayward, CA, USA) were used to determine the microbial CLPP following the procedure adapted from Garland and Mills [29]. The CLPP was measured by absorbance in each microplate well after incubation time, and it was determined by the average well-color development (AWCD) value. The AWCD was used to reflect the overall carbon source metabolic activity of microbes [29], which was calculated by using the following equation [30]:

$$AWCD = \sum (C_i - R) / n \quad (1)$$

where C_i is the difference in optical density value from each reacted-well between 590 and 750 nm, and R is the optical density (OD) value from the control well.

The changes of CLPP were determined by diversity parameters, such as indices of Shannon's diversity (H), substrate evenness (E), and substrate richness (S) indices [31]. The data of AWCD after 96 h of incubation from the Biolog EcoPlates [32] were used to calculate the microbes diversity indices as follows [29,33]:

$$H = - \sum P_i \times \ln P_i \quad (2)$$

$$E = H / \ln S \quad (3)$$

$$S = \text{Counts of all positive OD readings} \quad (4)$$

where P_i is the ratio of corrected absorbance value of each well to sum of absorbance value of all wells.

2.6. Statistical Analysis

One-way analysis of variance (ANOVA) was conducted using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA), and the cropping system was used as fixed factor, while block effect as a random factor. Prior to analysis, the normal distribution and the homoscedasticity of variance of the model residuals were checked, and data that failed the test were log-transformed to ensure the homogeneity of variances. Post hoc comparisons of means were completed with Duncan's multiple range test (Duncan) at the 0.05 level of probability [34]. AWCD was further analyzed using principal component analysis (PCA). Substrate utilization assay data were divided into six groups and the average absorbance per category was analyzed [33]. All meaningful loadings ($|r| > 0.6$) from PCA were considered significant and included in the interpretation of principal components [35]. Spearman correlations were used to examine the relationship between indices of soil microbial communities and soil C and N content.

3. Results

3.1. Soil Total Organic Carbon, Labile Organic Carbon, and Microbial Biomass Carbon

The TOC and LOC contents varied significantly with cropping sequences. Significant differences in soil TOC concentrations were detected among treatments at both soil depths ($p < 0.05$) (Figure 1a). At topsoil (0–30 cm), TOC was significantly greater in CA than in AP treatment, while at subsoil (30–60 cm) TOC was significantly greater in CA than in AFW and AP. The LOC at 0–30 cm was significantly greater in CA than in other cropping sequences, except the AF (Figure 1b). At 30–60 cm LOC was significantly greater in CA than in AFW and AP. The effects of cropping sequences on MBC were similar to, though somewhat different from, those on LOC. Consistent with LOC, the MBC of CA was greatest among treatments. At 0–30 cm, MBC was significantly greater in CA than in AFC, AP and AM, and at 30–60 cm, was significantly greater in CA than in AP (Figure 1c).

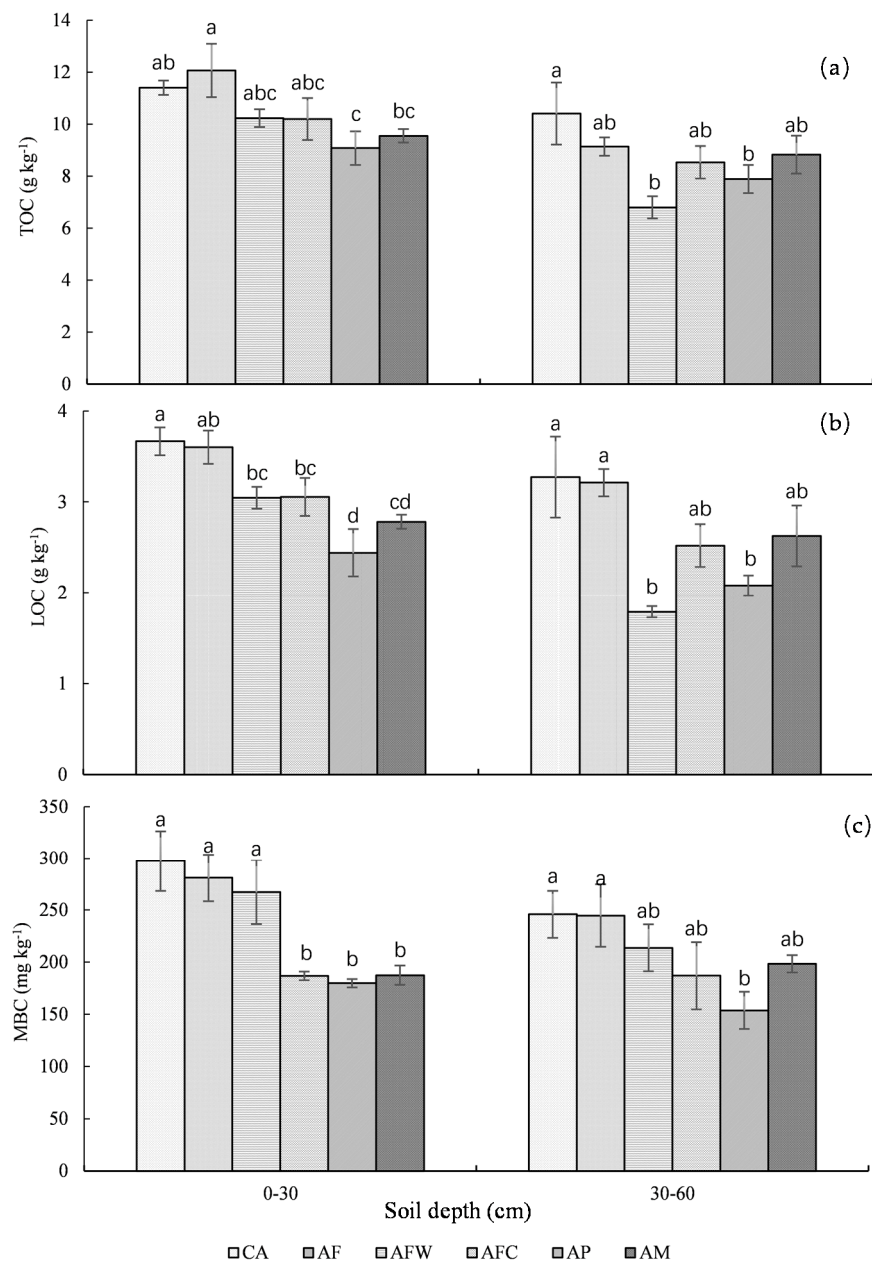


Figure 1. Soil organic carbon content at two soil depths in different cropping systems: (a) Soil total organic carbon (TOC); (b) Soil labile organic carbon (LOC); and (c) Soil microbial biomass carbon (MBC). Continuous alfalfa (CA), alfalfa-fallow (AF), alfalfa-fallow-wheat (AFW), alfalfa-fallow-maize (AFC), alfalfa-potato (AP), alfalfa-millet (AM). Error bars with different letters within a same soil depth indicate a significant difference at $p < 0.05$.

3.2. Soil Total Nitrogen and Microbial Biomass Nitrogen

Significant differences in soil TN concentrations were detected among treatments in topsoil (0–30 cm) ($p < 0.05$) (Figure 2a). The TN was significantly greater in CA than in other cropping sequences at 0–30 cm depth. However, TN was not influenced by cropping sequences at 30–60 cm depth. There were significant differences in soil MBN concentrations among treatments at both soil depths ($p < 0.05$) (Figure 2b). The MBN was significantly greater in CA, AFC, and AP treatments than in AF, AFW and AM treatments at 0–30 cm depth. The result at subsoil was consistent with the topsoil.

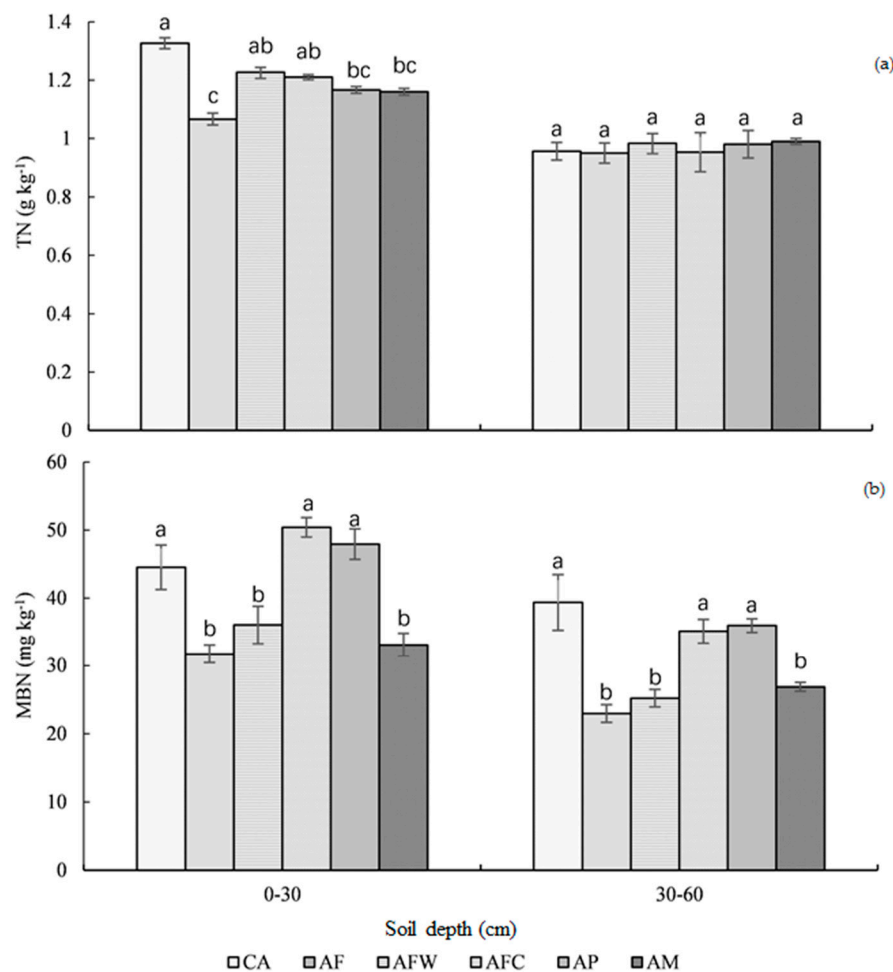


Figure 2. Soil nitrogen content at two soil depths in different cropping systems: (a) Soil total nitrogen (TN); (b) Soil microbial biomass nitrogen (MBN). Continuous alfalfa (CA), alfalfa-fallow (AF), alfalfa-fallow-wheat (AFW), alfalfa-fallow-maize (AFC), alfalfa-potato (AP), alfalfa-millet (AM). Error bars with different letters within a same soil depth indicate a significant difference at $p < 0.05$.

3.3. Community-Level Physiological Profiling

The AWCD is a good measure of the CLPP of the microbial community present in the different samples tested. As expected, AWCD increased with the incubation period (Figure 3), while it decreased with increasing soil depth across the different treatments. There were no significant changes of the AWCD values from 0 to 24 h. From 24 to 168 h, the soil microbes gradually adapted to the substrate environment in the microplate, and grew rapidly exponentially, and from 168 to 240 h, the AWCD values increased slowly and the microbial growth entered a steady phase. In the topsoil, the AWCD values were markedly greater with the CA and AF than the other treatments throughout the sampling period (Figure 3a). In the subsoil, the greatest AWCD value was found for the AFC, while AP constantly had the lowest AWCD throughout the sampling period. The AWCD value of the CA was greater than that of the AP and AM, but less than that of the AFC, AF, and AFW (Figure 3b).

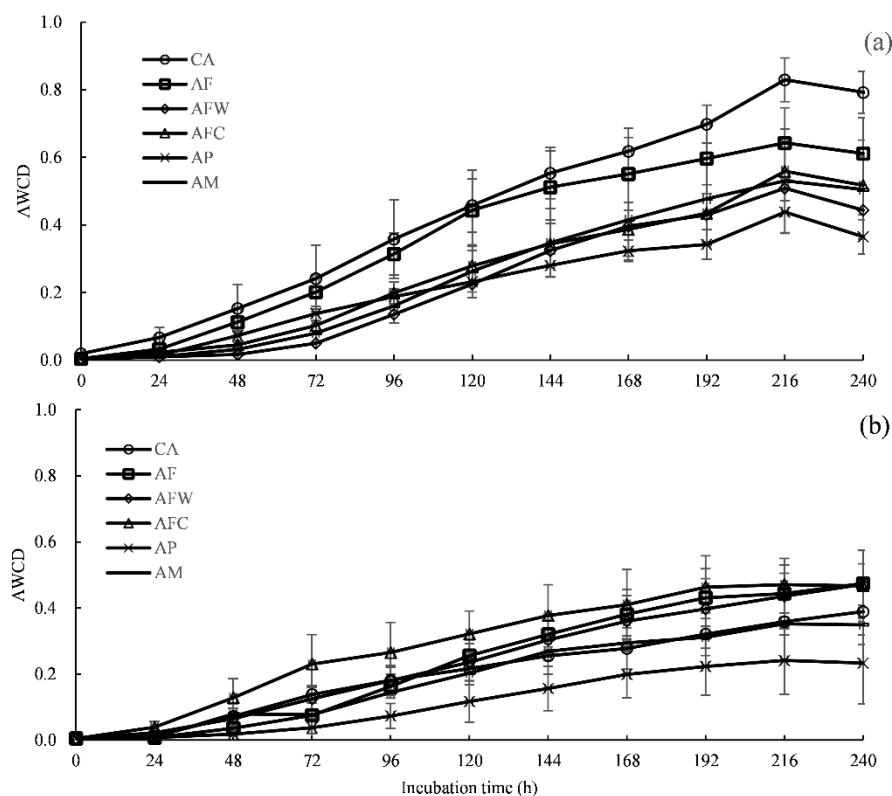


Figure 3. Average well-color development (AWCD) of soil microbial communities with incubation time in the topsoil (a, 0–30 cm) and subsoil (b, 30–60 cm) depth. Continuous alfalfa (CA), alfalfa-fallow (AF), alfalfa-fallow-wheat (AFW), alfalfa-fallow-maize (AFC), alfalfa-potato (AP), alfalfa-millet (AM).

In the topsoil, soil microbe functional diversity index (Shannon-Wiener diversity index) and the substrate richness index (number of substrates utilized) were significantly affected by cropping system ($p < 0.05$), being greatest in CA and lowest in AP (Table 2). That difference, however, was not observed in substrate evenness. Similar trends were found in the subsoil, but only the substrate richness index showed significant differences between the treatments ($p < 0.05$).

Table 2. Diversity indices of soil microbial communities (mean \pm se) in different cropping systems after 96 h of incubation.

Soil Depth	Treatment	Shannon's Diversity	Substrate Evenness	Substrate Richness
0–30 cm	CA	3.14 \pm 0.08a	0.93 \pm 0.02a	29.33 \pm 0.33a
	AF	2.95 \pm 0.12ab	0.91 \pm 0.02a	26.00 \pm 3.05a
	AFW	2.99 \pm 0.03ab	0.89 \pm 0.02a	28.33 \pm 1.20a
	AFC	2.75 \pm 0.22bc	0.84 \pm 0.05a	26.33 \pm 2.33a
	AP	2.55 \pm 0.10c	0.88 \pm 0.02a	18.33 \pm 0.88b
	AM	2.94 \pm 0.02ab	0.88 \pm 0.01a	28.00 \pm 0.58a
30–60 cm	CA	2.67 \pm 0.07a	0.87 \pm 0.01a	21.33 \pm 0.88a
	AF	2.55 \pm 0.3a	0.83 \pm 0.11a	21.33 \pm 0.33a
	AFW	2.56 \pm 0.20a	0.94 \pm 0.04a	15.33 \pm 1.86b
	AFC	2.53 \pm 0.23a	0.83 \pm 0.06a	20.67 \pm 1.33a
	AP	2.41 \pm 0.25a	0.88 \pm 0.09a	15.67 \pm 0.33b
	AM	2.69 \pm 0.12a	0.91 \pm 0.01a	19.67 \pm 1.85a

The mean value in each column within a same soil depth followed by different letters are different ($p < 0.05$). Continuous alfalfa (CA), alfalfa-fallow (AF), alfalfa-fallow-wheat (AFW), alfalfa-fallow-maize (AFC), alfalfa-potato (AP), alfalfa-millet (AM).

Principal component analysis (PCA) showed distinct differences among treatments (Figure 4). The proportion of variation explained by PC1 was 74% at 0–30 cm soil depth and 69% at 30–60 cm, respectively. Principal component loadings, comprising six categories of Biolog EcoPlates substrates, contributed towards the spread of variables along PC1 and PC2. In the topsoil, microorganisms that utilize carbohydrates, amino acids, carboxylic acids, polymers, miscellaneous and amines, influenced the spread of treatments along PC1 axis, whereas, those that breakdown carbohydrates, carboxylic acids, and polymers influenced differences along PC2 axis (Table 3). Distinct patterns were visible in topsoil—for the most part similar treatments clustered around each other in topsoil with exception of AFC (Figure 4a), with PC2 separating CA, AF and AP, AM, while AF and the other treatments were widely separated from each other on PC1 axis. However, PC1 could not separate continuous alfalfa and continuous annual cropping after alfalfa removed, with the exception of AM. In the subsoil, microorganisms that metabolize carbohydrates, amino acids, carboxylic acid, polymers, miscellaneous, and amines, influenced segregation of treatments on PC1 axis; however, only amino acids (Glycyl-L-glutamic acid) significantly influenced treatment difference along PC2 axis (Table 3). The spatial pattern of treatments was mixed in the subsoil, with continuous alfalfa placed far apart from all other treatments (Figure 4b). Both PC1 and PC2 separated treatments in both layers, but the degree of separation varied.

Table 3. Correlation analysis of carbon source utilization using the Biolog EcoPlates, versus the loading values of PC1 and PC2.

Carbon Source	Substrate	0–30 cm Soil Depth		30–60 cm Soil Depth	
		PC1	PC2	PC1	PC2
Carbohydrates	β -methyl-D-glucoside	0.897	−0.162	0.659	−0.574
	D-galactonic acid lactone	0.833	−0.338	0.832	−0.371
	D-xylose	0.767	−0.162	0.228	0.487
	D-erythritol	0.718	0.385	0.554	0.084
	D-mannitol	0.908	0.017	0.950	−0.069
	N-acetyl-D-glucosamine	0.938	−0.102	0.900	−0.317
	D-cellobiose	0.874	−0.032	0.810	−0.454
	α -D-glucose-1-phosphate	0.849	−0.250	0.788	−0.489
	α -D-lactose	0.251	0.690	−0.075	−0.038
	D,L- α -glycerol phosphate	0.711	−0.269	0.202	0.116
Amino acids	L-arginine	0.663	−0.009	0.419	0.584
	L-asparagine	0.943	0.043	0.869	0.207
	L-phenylalanine	0.870	−0.058	0.020	0.580
	L-serine	0.808	−0.011	0.795	0.209
	L-threonine	0.317	0.278	0.316	0.466
Carboxylic acids	Glycyl-L-glutamic acid	0.566	0.166	0.471	0.701
	Pyruvic acid methyl ester	0.437	0.532	0.567	0.481
	D-galacturonic acid	0.685	−0.306	0.740	−0.112
	γ -hydroxybutyric acid	0.543	0.123	0.352	0.191
	D-glucosaminic acid	0.824	−0.311	0.323	0.567
	Itaconic acid	0.614	0.133	−0.076	0.430
	α -ketobutyric acid	−0.044	0.700	−0.047	−0.190
Polymers	D-malic acid	0.803	0.025	−0.007	−0.077
	Tween 40	0.588	0.214	0.833	0.262
	Tween 80	0.695	0.356	0.748	0.214
	α -cyclodextrin	0.538	0.730	0.623	−0.141
	Glycogen	0.549	0.503	0.752	−0.014
Miscellaneous	2-hydroxybenzoic acid	0.756	−0.046	0.228	0.495
	4-hydroxybenzoic acid	0.806	0.054	0.690	0.269
Amines	Phenylethylamine	0.823	−0.321	0.515	−0.295
	Putrescine	0.710	−0.333	0.880	−0.359

The relation between indices of soil microbial communities and soil C and N was also analyzed. Both of Shannon's diversity and substrate richness were significantly correlated with TOC, LOC, MBC and TN (Table 4). However, MBN has no significant correlation with indices of soil microbial communities.

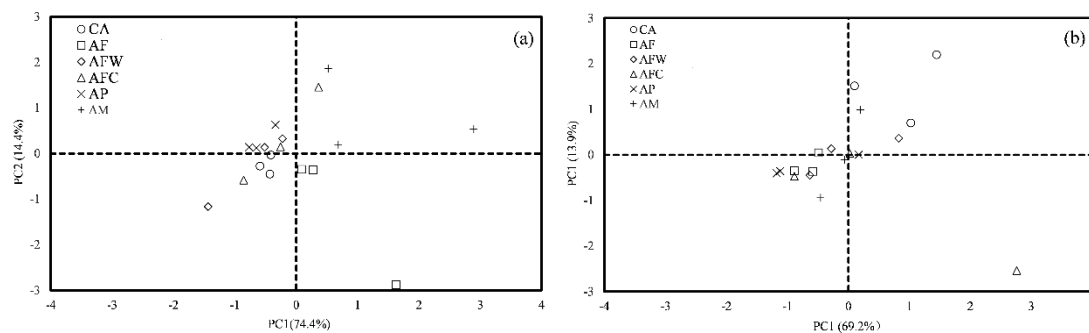


Figure 4. Principal component analysis (PCA) of the C substrates utilization pattern of soil microbes in the topsoil (a, 0–30 cm) and subsoil (b, 30–60 cm) depth in continuous alfalfa and its succession cropping systems. Continuous alfalfa (CA), alfalfa-fallow (AF), alfalfa-fallow-wheat (AFW), alfalfa-fallow-maize (AFC), alfalfa-potato (AP), alfalfa-millet (AM).

Table 4. Spearman's correlation between indices of soil microbial communities and soil C and N at 0–60 cm soil depth.

Measurement	Shannon's Diversity Index	Substrate Evenness	Substrate Richness
TOC	0.338 *	−0.158	0.535 **
LOC	0.337 *	−0.066	0.511 **
MBC	0.468 **	0.293	0.386 *
TN	0.425 **	0.09	0.517 **
MBN	0.051	−0.245	0.239

Note: *p* value significant at the <0.05 level * and 0.01 level ** (*n* = 36). Total organic carbon (TOC), labile organic carbon (LOC), microbial biomass carbon (MBC), total nitrogen (TN), and microbial biomass nitrogen (MBN).

4. Discussion

4.1. Soil C and N

It has been reported that soil organic carbon (SOC) levels normally do not change after 5–8 years of tillage and cropping sequence under dryland cropping systems [36–39]. However, in this study, SOC was greater in CA than in other cropping systems after four years. This could be the result of greater underground biomass C and a relatively undisturbed soil condition in CA. Tillage reduces SOC [36,40], while no-till improves C storage through reduced soil disturbance and slow decomposition of SOC [41]. Several studies have also reported that SOC was greater in alfalfa than in annual crops or greater in cropping systems containing alfalfa with cereals than in continuous cereal cropping [42–44]. Alfalfa was grown in an undisturbed soil condition, whereas plots in other cropping systems were tilled twice a year for planting and to control weeds from 2012 to 2016, except in the alfalfa-fallow system. A lower SOC in AP was likely due to the removal of potatoes at harvest, which caused more disruption and left less residues, such as roots in the soil profile than in the cropping systems, including cereal crops.

In general, soils in grassland-based cropping systems contain a higher proportion of N fractions [45,46]. Our results are consistent with the earlier findings. In the present study, CA significantly increased the soil TN content at 0–30 cm, which may be due to the biological N₂ fixation of alfalfa, root decomposition, and aboveground residues. The TN levels below the 30-cm depth, however, were not altered by cropping systems.

Similar to TOC, LOC and MBC contents varied with cropping sequences. The greater LOC and MBC in CA than in other treatments, indicate that perennial legume forages, such as alfalfa, increased soil microbial biomass and activity compared with annual crops, probably due to increased root biomass and rhizodeposited-C input, followed by a relatively undisturbed soil condition and continuous root growth throughout the year. Factors that may be responsible for the significant increase of LOC associated with alfalfa include large amounts of organic materials from alfalfa surface litter,

roots, and its exudates, usually greater than that of annual crops that are incorporated into soil and contribute to the increase in LOC [47]. Sainju et al. [48,49] found that perennial legume forages, such as rhizoma peanut (*Arachis glabrata* Benth.), increased MBC at 0–90 cm compared with perennial weeds due to greater root growth. Wu et al. [50] reported that the MBC amounts in alfalfa were higher than in cropland. Moore, Susanne and Tabatabai [45] found that MBC values were higher in multicropping systems taken in alfalfa meadow than in continuous maize systems. These observations all indicate that the establishment of perennial pastures or the introduction of alfalfa into crop-forage systems promotes soil biological activities and the turnover of soil nutrients.

4.2. Community-Level Physiological Profiling

The CLPP approach provide a more sensitive and ecologically meaningful measure of heterotrophic microbial community structure [29]. Soil microbial functional diversity was significantly influenced by soil type, climatic conditions, land use pattern, and their complex interactions, indicated by C substrate utilization [51]. Different cultivation methods and crop species have varying effects on soil microbial communities [52]. Our data showed that the CLPP, indicated by C substrate utilization, was influenced by cropping systems. Lower level of C-substrate utilization pattern by alfalfa removed and rotated with annual crops correlates with lower microbial biomass in those systems. The Shannon–Wiener diversity index, substrate evenness index, and substrate richness index varied in both soil layers. There were significant differences in those indices in the topsoil with the exception of the substrate evenness index. Continuous alfalfa produced higher Shannon–Wiener diversity index and substrate richness index in the topsoil. This was probably because of the greater belowground biomass C of alfalfa serves as source material for microbes and the absence of tillage, which might have reduced the vulnerability of the microbial communities imposed by tillage. It has been shown that structure and metabolic diversity of soil microorganisms was influenced by soil management practices [53]. Essel et al. [54] reported no-till influenced bacterial species diversity through improved soil chemical properties, which have the potential to affect the habitat and activity of soil microbes. However, the intensification of the tillage practice decreases the microbial diversity [55]. The lower microbial activity and functional diversity observed in AP treatment. It showed that potato continuous cropping increased the number and diversity of fungal dominant communities, whereas the number of bacteria and actinomycetes decreased [56,57]. As it is well known that bacteria are the predominant microorganisms, a lower number of bacteria could decrease the microbial community diversity and richness. The lower content of organic matter (TOC, LOC and MBC) also contributes to the lower microbial community activity in AP treatment, for a great positive correlation between diversity and richness of microbial communities and organic matter was observed in this study.

The average well-color development (AWCD) is an index which reflects microbial density on a Biolog EcoPlate. A greater AWCD value indicates higher metabolic activity in the soil microbial communities [58,59]. In topsoil, continuous alfalfa and alfalfa-fallow treatments significantly increased AWCD. Biolog substrate utilization assay detects the copiotrophic, or the “r” selected bacteria, which would be rapidly affected by a high level of organic carbon and nitrogen due to the continuous alfalfa. No tillage systems have been previously reported to increase microbial diversity indices, possibly as a result of growth and development of diverse microorganisms [54]. The AWCD response in the subsoil showed signs of treatment convergence, except for AP treatment, which had significantly lower AWCD. This could be attributed to the low amount of C and N in the subsoil, and especially that TN levels below the 30 cm depth were not altered by cropping systems.

Principal component analysis in topsoil clearly separated the alfalfa-fallow from the other cropping systems, however, the analysis was able to provide a clear interpretation of how microorganisms were affected by continuous alfalfa and continuous annual cropping after alfalfa removed in subsoil. The PCA separation suggests significant impact of cropping systems on soil microbial activity and functional diversity. This is primarily due to differences in microbial substrate utilization in those treatments. Grayston et al. [60] believed that crop identity can affect soil microbial communities

distinguishable from each other, and the C substrate derived from different crop roots are available to varying degrees in the soil, which contribute to soil microbial communities.

5. Conclusions

Overall, our results demonstrate that cropping systems can change soil biological activity. Soil biological properties, such as TOC, LOC, MBC, MBN, and microbial functional diversity can be used as indicators of management-induced changes to soil quality. For most soil biological properties evaluated, continuous alfalfa and continuous annual cropping after alfalfa did not lead to major differences in subsoil; however, cropping systems significantly altered various biological parameters in topsoil. Continuous alfalfa maintained greater soil C and N, and had a positive impact on soil microbial functional diversity. The results highlight greater microbial activity in soils with a perennial legume. The greater soil organic matter content and functional diversity of soil microbial CLPP in CA systems give the evidence that the soil nutrition and activity of microbes was not the limitation of the alfalfa development in the dryland area. The lower microbial activity and functional diversity observed in the potato field indicates the importance of crop selection in cropping systems, which provide a strategy for farmers to enhance the sustainability of farming system through soil microbial communities' management. This study confirms the importance of including alfalfa in their operation to improve soil health. Farmers should be encouraged to consider this crop in their cropping systems. Investigation of the factors other than soil organic C and microbial communities that contribute to the continuous crop productivity may further enhance cropping systems sustainability in dryland environments.

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References

1. Gan, Y.; Hamel, C.; O'Donovan, J.T.; Cutforth, H.; Zentner, R.P.; Campbell, C.A.; Niu, Y.; Poppy, L. Diversifying crop rotations with pulses enhances system productivity. *Sci. Rep.* **2015**, *5*, 14625. [[CrossRef](#)] [[PubMed](#)]
2. Niu, Y.; Bainard, L.D.; Bandara, M.; Hamel, C.; Gan, Y. Soil residual water and nutrients explain about 30% of the rotational effect in 4-yr pulse-intensified rotation systems. *Can. J. Plant. Sci.* **2017**, *97*, 852–864. [[CrossRef](#)]
3. Yang, X.M.; Kay, B.D. Rotation and tillage effects on soil organic carbon sequestration in a typical Hapludalf in Southern Ontario. *Soil Tillage Res.* **2001**, *59*, 107–114. [[CrossRef](#)]
4. Whitbread, A.M.; Blair, G.J.; Lefroy, R.D.B. Managing legume leys, residues and fertilisers to enhance the sustainability of wheat cropping systems in Australia: 2. Soil physical fertility and carbon. *Soil Tillage Res.* **2000**, *54*, 77–89. [[CrossRef](#)]
5. McCallum, M.H.; Connor, D.J.; O'Leary, G.L. Water use by lucerne and effect on crops in the Victorian Wimmera. *Aust. J. Agric. Res.* **2001**, *52*, 193–201. [[CrossRef](#)]
6. Fan, J.; Shao, M.; Wang, Q.; Jones, S.B.; Fu, X. Toward sustainable soil and water resources use in China's highly erodible semi-arid loess plateau. *Geoderma* **2010**, *155*, 93–100. [[CrossRef](#)]
7. Luo, Z.; Gan, Y.; Niu, Y.; Zhang, R.; Li, L.; Cai, L.; Xie, J. Soil quality indicators and crop yield under long-term tillage systems. *Exp. Agric.* **2017**, *53*, 497–511. [[CrossRef](#)]
8. Holford, I.C.R.; Schweitzer, B.E.; Crocker, G.J. Comparative effects of subterranean clover, medic, lucerne, and chickpea in wheat-rotations, on nitrogen, organic carbon, and moisture in two contrasting soils. *Aust. J. Soil Res.* **1998**, *36*, 57–72. [[CrossRef](#)]
9. McCallum, M.H.; Peoples, M.B.; Connor, D.J. Contributions of nitrogen by field pea (*Pisum sativum* L.) in a continuous cropping sequence compared with a lucerne (*Medicago sativa* L.)-based pasture ley in the Victorian Wimmera. *Crop. Pasture Sci.* **2000**, *51*, 13–22. [[CrossRef](#)]

10. Li, L.L.; Huang, G.B.; Zhang, R.Z.; Cai, L.Q.; Luo, Z.Z.; Jin, X.J.; Zhang, E.H.; Bill, B.; Murray, U. Effects of lucerne removal time on soil water and productivity in a lucerne-wheat rotation on the western Loess Plateau. *Acta Agron. Sin.* **2011**, *37*, 686–693. [\[CrossRef\]](#)
11. Li, Y.S. Productivity dynamic of alfalfa and its effects on water eco-environment. *Acta Pedol. Sin.* **2002**, *39*, 404–411. [\[CrossRef\]](#)
12. Shen, Y.Y.; Li, L.L.; Chen, W.; Robertson, M.; Unkovich, M.; Bellotti, W.; Probert, M. Soil water, soil nitrogen and productivity of lucerne-wheat sequences on deep silt loams in a summer dominant rainfall environment. *Field Crops Res.* **2009**, *111*, 97–108. [\[CrossRef\]](#)
13. Luo, Z.; Li, L.; Niu, Y.; Cai, L.; Zhang, R.; Xie, J. Soil dryness characteristics of alfalfa cropland and optimal growth years of alfalfa on the Loess Plateau of central Gansu, China. *Chin. J. Appl. Ecol.* **2015**, *26*, 3059–3065.
14. Zhang, G.S.; Huang, G.B.; Zhang, R.Z.; Huang, P.; Jin, X.J.; Li, L.L. The effects of lucerne on top soil properties of Huangmian soil. *Acta Prataculturae Sin.* **2003**, *12*, 88–93.
15. Li, J.; Chen, B.; Li, X.F.; Cheng, J.M.; Hao, M.D. Effects of deep soil desiccations on alfalfa grasslands in different rainfall areas of the Loess Plateau of China. *Acta Ecol. Sin.* **2007**, *27*, 75–89.
16. Song, L.P.; Luo, Z.Z.; Li, L.L.; Cai, L.Q.; Zhang, R.Z.; Niu, Y.N. Effect of lucerne-crop rotations on soil physical properties in the semi-arid Loess Plateau of Central Gansu. *Acta Prataculturae Sin.* **2015**, *24*, 12–20.
17. Kotroczo, Z.; Juhos, K.; Biró, B.; Kocsis, T.; Pabar, S.; Fekete, I.; Varga, C. Effect of detritus manipulation on different organic matter decompositions in temperate deciduous forest soils. *Forests* **2020**, *11*, 675. [\[CrossRef\]](#)
18. Lehman, R.; Cambardella, C.; Stott, D.; Acosta-Martinez, V.; Manter, D.; Buyer, J.; Maul, J.; Smith, J.; Collins, H.; Halvorson, J.; et al. Understanding and enhancing soil biological health: The solution for reversing soil degradation. *Sustainability* **2015**, *7*, 988–1027. [\[CrossRef\]](#)
19. Gessner, M.O.; Swan, C.M.; Dang, C.K.; McKie, B.G.; Bardgett, R.D.; Wall, D.H.; Hattenschwiler, S. Diversity meets decomposition. *Trends Ecol. Evol.* **2010**, *25*, 372–380. [\[CrossRef\]](#)
20. Liu, X.; Jiang, Q.; Hu, X.; Zhang, S.; Liu, Y.; Huang, W.; Ding, W. Soil microbial carbon metabolism reveals a disease suppression pattern in continuous ginger mono-cropping fields. *Appl. Soil Ecol.* **2019**, *144*, 165–169. [\[CrossRef\]](#)
21. Nayyar, A.; Hamel, C.; Lafond, G.; Gossen, B.D.; Hanson, K.; Germida, J. Soil microbial quality associated with yield reduction in continuous-pea. *Appl. Soil Ecol.* **2009**, *43*, 115–121. [\[CrossRef\]](#)
22. Yusuf, A.A.; Abaidoo, R.C.; Iwuafor, E.N.O.; Olufajo, O.O.; Sanginga, N. Rotation effects of grain legumes and fallow on maize yield, microbial biomass and chemical properties of an Alfisol in the Nigerian savanna. *Agric. Ecosyst. Environ.* **2009**, *129*, 325–331. [\[CrossRef\]](#)
23. Niu, Y.; Bainard, L.D.; May, W.E.; Hossain, Z.; Hamel, C.; Gan, Y. Intensified pulse rotations buildup pea rhizosphere pathogens in cereal and pulse based cropping systems. *Front. Microbiol.* **2018**, *9*. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Wang, Z.Y.; Wang, Y.X.; Chen, Z.R. The nature of soybean-cropping. *Soybean Sci.* **1991**, *10*, 31–36.
25. Lamptey, S.; Li, L.; Xie, J.; Zhang, R.; Luo, Z.; Cai, L.; Liu, J. Soil respiration and net ecosystem production under different tillage practices in semi-arid Northwest China. *Plant. Soil Environ.* **2017**, *63*, 14–21. [\[CrossRef\]](#)
26. Lefroy, R.D.B.; Blair, G.J.; Strong, W.M. Changes in soil organic matter with cropping as measured by organic carbon fractions and ¹³C natural isotope abundance. *Plant. Soil* **1993**, *155/156*, 399–402. [\[CrossRef\]](#)
27. Vance, E.D.; Brookes, P.C.; Jenkinson, D.S. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* **1987**, *19*, 703–707. [\[CrossRef\]](#)
28. Brookes, P.C.; Landman, A.; Pruden, G.; Jenkinson, D.S. Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biol. Biochem.* **1985**, *17*, 837–842. [\[CrossRef\]](#)
29. Garland, J.L.; Mills, A.L. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon source utilization. *Appl. Environ. Microbiol.* **1991**, *57*, 2351–2359. [\[CrossRef\]](#)
30. Classen, A.T.; Boyle, S.I.; Haskins, K.E.; Overby, S.T.; Hart, S.C. Community-level physiological profiles of bacteria and fungi: Plate type and incubation temperature influences on contrasting soils. *FEMS Microbiol. Ecol.* **2003**, *44*, 319–328. [\[CrossRef\]](#)
31. Kourtev, P.S.; Ehrenfeld, J.G.; Haggblom, M. Exotic plant species alter the microbial community structure and function in the soil. *Ecology* **2002**, *83*, 3152–3166. [\[CrossRef\]](#)

32. Zhang, Y.Y.; Qu, L.Y.; Chen, L.D. An amendment on information extraction of Biolog EcoPlate™. *Microbiology* **2009**, *36*, 1083–1091.
33. Zak, J.C.; Willig, M.R.; Moorhead, D.L.; Wildman, H.G. Functional diversity of microbial communities: A quantitative approach. *Soil Biol. Biochem.* **1994**, *26*, 1101–1108. [[CrossRef](#)]
34. Liu, Y.; Zhu, G.; Hai, X.; Li, J.; Shangguan, Z.; Peng, C.; Deng, L. Long-term forest succession improves plant diversity and soil quality but not significantly increase soil microbial diversity: Evidence from the Loess Plateau. *Ecol. Eng.* **2020**, *142*, 105631. [[CrossRef](#)]
35. Song, M.Y.; Li, Z.P.; Wu, M.; Liu, M.; Jiang, C.Y. Changes in soil microbial biomass and community structure with cultivation chronosequence of greenhouse vegetables. *Sci. Agric. Sin.* **2015**, *48*, 3635–3644.
36. Halvorson, A.D.; Peterson, G.A.; Reule, C.A. Tillage system and crop rotation effects on dryland crop yields and soil carbon in the central Great Plains. *Agron. J.* **2002**, *94*, 1429–1436. [[CrossRef](#)]
37. Halvorson, A.D.; Wienhold, B.J.; Black, A.L. Tillage, nitrogen, and cropping system effects on soil carbon sequestration. *Soil Sci. Soc. Am. J.* **2002**, *66*, 906–912. [[CrossRef](#)]
38. Ortega, R.A.; Peterson, G.A.; Westfall, D.G. Residue accumulation and changes in soil organic matter as affected by cropping intensity in no-till dryland agroecosystem. *Agron. J.* **2002**, *94*, 944–954. [[CrossRef](#)]
39. Sainju, U.M.; Lenssen, A.W.; Caesar-TonThat, T.; Waddell, J. Tillage and crop rotation effects on dryland soil and residue carbon and nitrogen. *Soil Sci. Soc. Am. J.* **2006**, *70*, 668–678. [[CrossRef](#)]
40. Schomberg, H.H.; Jones, O.R. Carbon and nitrogen conservation in dryland tillage and cropping systems. *Soil Sci. Soc. Am. J.* **1999**, *63*, 1359–1366. [[CrossRef](#)]
41. Andruschkewitsch, R.; Geisseler, D.; Koch, H.J.; Ludwig, B. Effects of tillage on contents of organic carbon, nitrogen, water-stable aggregates and light fraction for four different long-term trials. *Geoderma* **2013**, *192*, 368–377. [[CrossRef](#)]
42. Russell, A.E.; Laird, D.A.; Parkin, T.B.; Mallarino, A.P. Impact of nitrogen fertilization and cropping system on carbon sequestration in midwestern mollisols. *Soil Sci. Soc. Am. J.* **2005**, *69*, 413–422. [[CrossRef](#)]
43. Syswerda, S.P.; Corbin, A.T.; Mokma, D.J.; Kravchenko, A.N.; Robertson, G.P. Agricultural management and soil carbon storage in surface vs. deep layers. *Soil Sci. Soc. Am. J.* **2011**, *75*, 92–101. [[CrossRef](#)]
44. VandenBygaart, A.J.; Bremer, E.; McConkey, B.G.; Ellert, B.H.; Angers, D.A.; Carter, M.R.; Drury, C.F.; Lafond, G.P.; McKenzie, R.H. Impact of sampling depth on differences in soil carbon stocks in long-term agroecosystem experiments. *Soil Sci. Soc. Am. J.* **2011**, *75*, 226–234. [[CrossRef](#)]
45. Moore, J.M.; Susanne, K.; Tabatabai, M.A. Soil microbial biomass carbon and nitrogen as affected by cropping systems. *Biol. Fert. Soils* **2000**, *31*, 200–210. [[CrossRef](#)]
46. Peoples, M.B.; Bowman, A.M.; Gault, R.R.; Herridge, D.F.; McCallum, M.H.; McCormick, K.M.; Norton, R.M.; Rochester, I.J.; Scammell, G.J.; Schwenke, G.D. Factors regulating the contributions of fixed nitrogen by pasture and crop legumes to different farming systems of eastern Australia. *Plant Soil* **2001**, *228*, 29–41. [[CrossRef](#)]
47. Paustian, K.; Andren, O.; Janzen, H.H.; Lal, R.; Smith, P.; Tian, G.; Tiessen, H.; Van Noordwijk, M.; Woomer, P.L. Agricultural soils as a sink to mitigate CO₂ emissions. *Soil Use Manag.* **1997**, *13*, 230–244. [[CrossRef](#)]
48. Sainju, U.M.; Terrill, T.H.; Gelaye, S.; Singh, B.P. Soil aggregation and carbon and nitrogen pools under rhizoma peanut and perennial weeds. *Soil Sci. Soc. Am. J.* **2003**, *67*, 146–155. [[CrossRef](#)]
49. Sainju, U.M.; Terrill, T.H.; Gelaye, S.; Singh, B.P. Soil carbon and nitrogen pools under long-term productivity of rhizoma peanut and perennial weeds management systems. *Arch. Agron. Soil Sci.* **2006**, *52*, 45–59. [[CrossRef](#)]
50. Wu, T.Y.; Schoenau, J.J.; Li, F.M.; Qian, P.Y.; Malhi, S.S.; Shi, Y.C. Effect of tillage and rotation on organic carbon forms of chernozemic soils in Saskatchewan. *J. Plant. Nutr. Soil Sci.* **2003**, *166*, 328–335. [[CrossRef](#)]
51. Luo, Q.; Huang, B.L.; Tang, Z.X.; Lai, L.M.; Wei, W. Carbon source utilization of microbes in saline soil of three vegetation types in Xinjiang, China. *Chin. J. Appl. Environ. Biol.* **2013**, *19*, 96–104. [[CrossRef](#)]
52. Buenemann, E.K.; Bossio, D.A.; Smithson, P.C.; Frossard, E.; Oberson, A. Microbial community composition and substrate use in a highly weathered soil as affected by crop rotation and P fertilization. *Soil Biol. Biochem.* **2004**, *36*, 889–901. [[CrossRef](#)]
53. Bending, G.D.; Turner, M.K.; Jones, J.E. Interaction between crop residue and soil organic matter quality and the functional diversity of soil microbial communities. *Soil Biol. Biochem.* **2002**, *34*, 1073–1082. [[CrossRef](#)]

54. Essel, E.; Li, L.; Deng, C.; Xie, J.; Zhang, R.; Luo, Z.; Cai, L. Evaluation of bacterial and fungal diversity in a long-term spring wheat–field pea rotation field under different tillage practices. *Can. J. Soil Sci.* **2018**, *98*, 619–637. [[CrossRef](#)]
55. Silva, A.P.; Babujia, L.C.; Matsumoto, L.S.; Guimarães, M.F.; Hungri, M. Bacterial diversity under different tillage and crop rotation systems in an oxisol of Southern Brazil. *Open Agric. J.* **2013**, *7*, 40–47. [[CrossRef](#)]
56. Meng, P.P.; Liu, X.; Qiu, H.Z.; Zhang, W.M.; Zhang, C.H. Fungal population structure and its biological effect in rhizosphere soil of continuously cropped potato. *Chin. J. Appl. Ecol.* **2012**, *23*, 3079–3086.
57. Qin, S.; Yeboah, S.; Xu, X.; Liu, Y.; Yu, B. Analysis on fungal diversity in rhizosphere soil of continuous cropping potato subjected to different furrow-ridge mulching managements. *Front. Microbiol.* **2017**, *8*, 845. [[CrossRef](#)]
58. Garland, J.L. Analysis and interpretation of community-level physiological profiles in microbial ecology. *FEMS Microbiol. Ecol.* **1997**, *24*, 289–300. [[CrossRef](#)]
59. Konopka, A.; Oliver, L.; Turco, R.F. The use of carbon substrate utilization patterns in environmental and ecological microbiology. *Microb. Ecol.* **1998**, *35*, 103–115. [[CrossRef](#)]
60. Grayston, S.J.; Wang, S.Q.; Campbell, C.D.; Edwards, A.C. Selective influence of plant species on microbial diversity in the rhizosphere. *Soil Biol. Biochem.* **1998**, *30*, 369–378. [[CrossRef](#)]



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