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Time-course of soil microbial communities in different tillage and crop rotation systems

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ABSTRACT

The soil microbial communities involved in the biogeochemical cycles of plant nutrients are negatively affected by unfavorable agricultural practices. In three tillage systems (traditional tillage, TT; traditional tillage with residue incorporation, TTI; and conservation tillage, CT) with three crop rotations (cereal-cereal, C-C; legume-cereal, L-C; and cereal-legume, C-L) at three soil depths (0-5, 5-15, and 15-30 cm), the effects on the populations of bacteria, actinomycetes, and fungi over a period of six crop cycles (3 yr) were evaluated. The tillage system, crop rotation, and depth affected the concentration of microbes in the soil. Under TT/C-C (regional control), they decreased by 7.5%; in contrast, under CT/L-C and TTI/L-C, they increased by 144% and 76%, respectively. Regardless of the tillage system, rotation with legumes, especially when the legume was cultivated in the spring-summer cycle (C-L), caused significant increases in microbial populations. At the end of 3 yr, under CT and TTI the populations of actinomycetes increased, while the fungal population remained stable and the bacterial populations fluctuated in the different crop cycles. In all treatments, the concentration of microorganisms decreased with soil depth. Local practices represent a risk to the diversity of soil microbiota, and it is imperative that farmers adopt conservation practices to achieve sustainability.

Key words: CFUs, rhizospheric microorganisms, soil degradation, Vertisol soil.

INTRODUCTION

In Mexico, there are 32.4 million ha of land for agricultural use; 44.9% shows some type of degradation, and 35% is associated with agricultural and livestock activities (SEMARNAT, 2016). Loss of fertility, pollution, and salinization are the most frequent types of soil degradation and are due to the excessive use of agrochemicals, unfavorable soil management practices, loss of vegetation cover, and decreased biological activity (Cotler et al., 2016).

Soil is an important C reserve that is potentially volatile, depending on the balance between photosynthesis, respiration, and stabilization. The mean C content in Mexican soils is 56.1 Mg ha^{-1} (Segura-Castruita et al., 2005). In addition, agricultural expansion and erosion increase C release, resulting in lower productivity and decreased biodiversity (Seymour and Harris, 2019). This balance, which is influenced by management practices and microbial communities, can determine the role of the soil as a reservoir or emitter of C (Trivedi et al., 2013). The metabolic diversity of these communities affects the formation of more stable recalcitrant compounds, which interact in different ways with the soil physical and chemical properties, although many indicators have been used to assess soil quality, the most sensitive indicators are biochemical attributes (Notaro et al., 2018).

To achieve sustainability, it is essential to consider the great microbial biodiversity harbored by the soil, which plays a significant role in the environmental services it provides and which has been mostly ignored in sustainable agriculture

research. Current practices represent a risk to the vast diversity of soil microbiota, a fact that must be considered if we are to move towards truly sustainable management (FAO, 2015).

In the central region of Mexico known as El Bajío (*ca*. 1.1 × 10^6 ha), intensive agriculture, traditional tillage, cereal-cereal rotation, residue burning or extraction, and poor irrigation water management have caused the loss of natural soil fertility and therefore the need to apply increasing doses of synthetic fertilizers (Grageda-Cabrera et al., 2011). Faced with this problem, it is imperative for farmers to adopt new soil and crop management practices through effective technology transfer without compromising productivity, and perhaps even increasing it (Garnett et al., 2013). For this reason, the objective of the present study was to quantify the microbial populations of bacteria, actinomycetes, and fungi under different tillage and crop rotation systems.

MATERIALS AND METHODS

A long-term experiment was conducted from June 2016 to April 2019 at Bajío Experimental Field (Campo Experimental Bajío) of the National Institute of Forest, Agricultural, and Livestock Research (Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, INIFAP) in Celaya (20°35'06.59'' N, 100°49'46.84'' W; 1769 m a.s.l.), Guanajuato, Mexico. The mean annual rainfall is 543.5 mm and the mean annual temperature is 18.1 °C (Table 1). The soil is classified as pelic Vertisol with a pH (1:2 water) of 7.47, organic matter content of 1.89%, and a clay loam texture (FAO, 2008).

The experimental design was strip-plots subdivided over time (Gomez and Gomez, 1984) with three replicates. The vertical strip consisted of three types of tillage: traditional tillage (TT), traditional tillage with residue incorporation (TTI), and conservation tillage (LC). The horizontal strip consisted of three types of crop rotation: cereal-cereal (C-C) (regional control), legume-cereal (L-C), and cereal-legume (C-L); the regional control was TT with C-C rotation. The third strip consisted of three soil depths: 0-5, 5-15, and 15-30 cm. Each experimental unit had an approximate area of 259.2 m^2. Agronomic management depended on the treatment; these practices were carried out according to the recommendations proposed by INIFAP. The experiment was performed over six crop cycles (two per year). In the spring-summer (S-S) cycles, the cereal was corn (*Zea mays* L.) and the legume was bean (*Phaseolus vulgaris* L.); in the autumn-winter (A-W) cycles, the cereal was wheat (*Triticum aestivum* L.) and the legume was chickpea (*Cicer arietinum* L.) The microbiological variables evaluated were the populations of bacteria, actinomycetes, and fungi. We quantified the colony forming units (CFUs) using the serial dilution and plate extension technique (Tortora et al., 2016). Soil was sampled in zig-zags at 12 points in each experimental unit at three depths: 0-5, 5-15, and 15-30 cm. Microbial populations were quantified at the beginning of the experiment and at the end of each of the six crop cycles; cycles 1, 3, and 5 corresponded to spring-summer (S-S), cycles 2, 4, and 6 to autumn-winter (A-W). The data were analyzed using the generalized linear model procedure and the principal component analysis procedure of SAS (SAS Institute, Cary, North Carolina, USA). Means were compared by Tukey's test when the F test was significant (HSD with $p \leq 0.05$).

RESULTS AND DISCUSSION

The ANOVA showed highly significant differences ($p \leq 0.01$) between the main sources of variation, i.e., tillage, rotation, depth, and crop cycles, as well as between their interactions in the populations of bacteria, actinomycetes, and total microbes (Table 2). In the individual sources of variation, the highly significant differences in order of magnitude for the bacterial populations were tillage, rotation, crop cycle, and depth; for the populations of actinomycetes, tillage,

Table 1. Maximum, minimum, and average temperatures and precipitations accumulated during the experimental period from July 2016 to June 2019 (weather station INIFAP-CEBAJ).

Crop cycles	Maximum temperature	Minimum temperature	Average temperature	Precipitation accumulated
		°C		mm
S-S 2016	30.4	3.3	29.7	311.8
A-W 2016-2017	34.8	0.2	31.1	98.0
S-S 2017	30.7	0.6	30.2	367.4
A-W 2017-2018	36.4	-1.0	31.3	303.6
S-S 2018	30.8	1.7	29.6	496.2
A-W 2018-2019	33.7	-5.0	31.2	99.2

S-S: Spring-summer; A-W: autumn-winter.

crop cycle, depth, and crop rotation; and for the fungal populations, depth alone. Among the interactions, the most important differences for bacteria were in Tillage×Depth and Tillage×Crop cycles; for actinomycetes, it was Tillage×Depth and Tillage×Crop cycle; and for fungi, it was Depth×Crop cycles and Tillage×Depth.

When we analyzed the types of tillage (Table 3), bacterial populations in TTI and CT were 24.41% and 39.22% higher than in TT. The greatest bacterial development was observed in CT. The agricultural residues left on the soil surface and the nonmoving of the soil helped not disturb the dynamics of these populations. The same behavior was observed in the populations of actinomycetes, where the increases in TTI and CT were 43.69% and 118.61% with respect to TT, probably because in CT, compounds with cellulose, hemicellulose, and lignin were found in a greater proportion due to the permanence of the residues, which are used by actinomycetes, causing their predominance in surfaces with agricultural residues (Bhatti et al., 2017). The fungal populations did not differ significantly by type of tillage, but a lower amount was observed in the TT treatment, which did not incorporate residues. Similar results were obtained by Afanador-Barajas et al. (2020), who found a significant effect of agricultural management on soil properties and the content of soil microorganisms (bacteria and actinomycetes). The type of tillage influences the microbiological variables; conservation systems promote microbial populations (Bu et al., 2020; Kraut-Cohen et al., 2020; Li et al., 2020; Sun et al., 2020).

Table 2. Mean squares, degrees of freedom and statistical significance of ANOVA for microbial populations at different tillage systems and crop rotation through six growing seasons at three soil depths.

		Mean square			
Source of variation	df	Bacteria	Actinomycetes	Fungi	Total
Replication	2	0.8083ns	0.5826ns	0.0003ns	2.4814ns
Tillage (T)	2	46.742**	161.767**	0.0009ns	373.926**
Error a	4	0.0822	0.5087	0.0009	0.6904
Crop rotation (R)	2	13.856**	20.091**	0.0017ns	54.702**
Error b	4	0.1883	0.1142	0.0008	0.2650
T×R	4	10.425**	13.729**	0.0015ns	48.276**
Error c	18	0.0665	0.1217	0.0004	0.3843
Soil depths (D)	2	2.799**	61.654**	0.0725**	68.771**
T×D	4	12.430**	35.721**	0.0167**	30.987**
R×D	4	10.171**	7.872**	0.0127**	27.424**
T×R×D	8	6.543**	9.865**	0.0245**	16.207**
Error d	36	0.181	0.248	0.0028	0.416
Crop cycles (C)	6	8.259**	71.796**	0.0014ns	67.714**
T×C	12	12.310**	28.299**	0.0022ns	25.055**
R×C	12	10.855**	11.602**	0.0009ns	5.340**
D×C	12	7.508**	13.424**	0.0285**	27.918**
T×R×C	24	6.302**	6.542**	0.0013ns	3.960**
T×D×C	24	8.972**	9.237**	0.0142**	17.756**
R×D×C	24	10.597**	8.855**	0.0100**	31.872**
T×R×D×C	48	12.425**	7.291**	0.0150**	24.157**
Error	324	0.1790	0.233	0.0017	0.496
CV, %		18.776	17.615	27.330	13.860

**Significant at the 0.01 probability level; ns: nonsignificant; df: degrees of freedom; CV: coefficient of variation.

Table 3. Microbial populations in soil under different tillage systems over a period of six crop cycles.

		Microbial populations		
Tillage	Bacteria	Actinomycetes	Fungi	Total
	CFUs × 10^5			
TT	5.2638c	5.4998c	0.454362a	11.2180c
TTI	6.7801b	7.6572b	0.461016a	14.8984b
CT	8.2475a	11.0078a	0.468142a	19.6353a
HSD	0.4349	0.5206	0.0260	0.8331

Means followed by different letter in each column are significantly different according to the HSD test ($p \leq 0.05$).
TT: Traditional tillage; TTI: traditional tillage with residue incorporation; CT: conservation tillage; CFUs: colony forming units.

Lower soil disturbance preserves microhabitats, creating a better environment for microbial growth (Zuber and Villamil, 2016; Li et al., 2018). In addition, the degree of disturbance also influences the microbial utilization rate of C and of other elements, which causes significant differences in microbial diversity and catabolic diversity (Wang et al., 2020). The type of tillage influences the physical properties of the soil (Hubbard et al., 2013), and these properties correlate with bacterial communities.

Table 4 shows the results of the microbial populations evaluated in the different crop rotations. The L-C rotation increased bacterial populations, while the C-L rotation increased the populations of actinomycetes. In contrast, the C-C rotation had the lowest amounts of bacteria and actinomycetes. Fungal populations were significantly similar between the rotations. The total populations increased when there was rotation with legumes. This pattern was also observed by Song et al. (2007), who, through various evaluation cycles, cultivated cereal with legume and concluded that intercropping promotes the development of bacterial populations. Each plant species produces different amounts and compositions of exudates, which promotes microorganism diversity and abundance (Merbach et al., 1999). The C-L and L-C rotations are favorable for soil management since they increase the development of microbial populations, possibly due to the types of organisms that develop in each type of crop. However, it is difficult for local farmers to consider this aspect since the economic gains from the sale of crops are greater with the C-C rotation.

In the evaluation of microbial populations at different depths, the number of microorganisms decreased as the depth in the soil profile increased (Table 5). However, the bacterial populations showed the same concentration in the 0-5 and 5-15 cm strata, while fungal populations were significantly equal between 5-15, and 15-30 cm. This pattern coincides with studies in which microbial biomass often decreases with soil depth (Hartmann et al., 2009; Zhang et al., 2019). Eilers et al. (2012), when analyzing soil microbial communities at different depths, found significant differences between profiles separated by 10 cm, and the same was observed in the present study. In addition, those authors highlighted that changes in environmental conditions with soil depth influenced microbial communities, with the most pronounced effects in the first 25 cm. The communities of microorganisms in the soil live predominantly aerobically; that is, they need oxygen for their metabolism, and oxygen decreases as the depth increases (Ball et al., 1990).

In the analysis of the microbial populations over the six crop cycles (Table 6), there were variations between cycles, which could have been due to the climatic conditions in each of them. In addition, each type of microorganism showed a different behavior. Considering the mean of all treatments, fungi were the most stable against these changes, and the general trend was an increase in microbial population over the course of the six crop cycles.

Table 4. Microbial populations in soil under different crop rotations over a period of six crop cycles.

Crop rotation	Microbial populations			
	Bacteria	Actinomycetes	Fungi	Total
	CFUs × 10^5			
C-C	5.9439c	7.0669c	0.466931a	13.3896b
L-C	7.5683a	8.0751b	0.450569a	16.0939a
C-L	6.7792b	9.0229a	0.466020a	16.2681a
HSD	0.4349	0.5206	0.0260	0.3707

Means followed by different letter in each column are significantly different according to the HSD test ($p \leq 0.05$).
C-C: Cereal-cereal rotation; L-C: legume-cereal rotation; C-L: cereal-legume rotation; CFUs: colony forming units.

Table 5. Microbial populations in soil under different soil depths over a period of six crop cycles.

Soil depths (cm)	Microbial populations			
	Bacteria	Actinomycetes	Fungi	Total
	CFUs × 10^5			
0-5	2.3657a	3.1709a	0.1748a	5.6641a
5-15	2.2735a	2.8280b	0.1501b	5.1264b
15-30	2.1245b	2.0558c	0.1361b	4.4599c
HSD	0.1391	0.1595	0.0172	0.2065

Means followed by different letter in each column are significantly different according to the HSD test ($p \leq 0.05$).
CFUs: Colony forming units.

Table 6. Microbial populations in each of six crop cycles.

Crop cycle	Bacteria	Microbial populations		
		Actinomycetes	Fungi	Total
	CFUs × 10^5			
IS	6.6366bc	4.0971e	0.4523a	11.1861d
1*	7.2142ab	5.4178d	0.4541a	12.8805c
2	7.2786ab	7.2510c	0.4853a	15.0150b
3	7.4019ab	7.3846c	0.4666a	15.2531b
4	4.8973d	9.7990b	0.4484a	15.1448b
5	6.2328c	11.9605a	0.4557a	18.6491a
6	7.6852a	10.4746b	0.4655a	18.6253a
HSD	0.8175	0.9786	0.0488	1.5659

Means followed by different letter in each column are significantly different according to the HSD test ($p \leq 0.05$).
*Sampling done after harvest.
CFUs: Colony forming units; IS: initial sampling; Cycles 1, 3, and 5 corresponded to spring-summer cycles 2, 4, and 6 to autumn-winter.

In the principal component analysis of the Tillage×Rotation interaction (Table 7), the most important variables were expressed in principal component 1 (PC1): bacteria, actinomycetes, and total microbes. PC2 contained only fungi, continuous cropping could increase the relative abundance of some fungi (Bai et al., 2018). Both principal components together explained 97.1% of the total variation.

Figure 1 shows the results of the principal component analysis of the Tillage×Rotation interaction, where three groups were formed. The first was characterized by the abundance of bacteria and actinomycetes, highlighting the CT/C-L and CT/L-C interactions, which shared in common the type of tillage and rotations that included legumes. At the opposite extreme, the group with the lowest population of bacteria and actinomycetes was that treated with TT regardless of the rotation used. In the central part of the graph, there is a group formed by TTI with its respective rotations and CT/C-C, which presented intermediate amounts of bacteria and actinomycetes. Within each group, differences were observed with respect to the fungal population; the group formed by CT/C-L and CT/L-C shows a greater amount of these microorganisms, and the results may be due to the type of residue, the time of application, and the C:N ratio (Martínez and Leyva, 2014). In CT/C-L, wheat straw, with a C:N ratio of 87, was added in the S-S cycle, and bean straw (C:N 17) was added in the A-W cycle. In CT/L-C, chickpea straw (C:N 21) was added in the S-S and corn straw (C:N 65) in the A-W. It is likely that the amount of added C promoted an increase in fungal populations, since these maintain a C:N ratio of 10:1, greater than that of bacteria (5:1) or actinomycetes (6:1) (Troeh and Thompson, 2005).

In the principal component analysis of the Tillage×Depth interaction, the most important variables were expressed in PC1: bacteria, fungi, and total microbes. PC2 was only actinomycetes. Both principal components together explained 92.1% of the total variation generated (Table 8).

Table 7. Characteristic vectors of the principal components for the microbial populations evaluated in the Tillage systems×Crop rotation interaction over a period of six crop cycles in the 0-30 cm soil profile.

Variable (microbial populations)	Characteristic vectors	
	PC1	PC2
Bacteria	0.544043	-0.236284
Actinomycetes	0.561397	-0.061493
Fungi	0.5613	0.96043
Total	0.571475	-0.134021
Characteristic value	3.008068	0.8791832
Explained variance, %	75.2	21.9

PC1: Principal component 1; PC2: Principal component 2.

Figure 1. Principal components analysis of microbial populations evaluated in soil under different tillage systems and crop rotation over period of six crop cycles in the 0-30 cm soil profile.

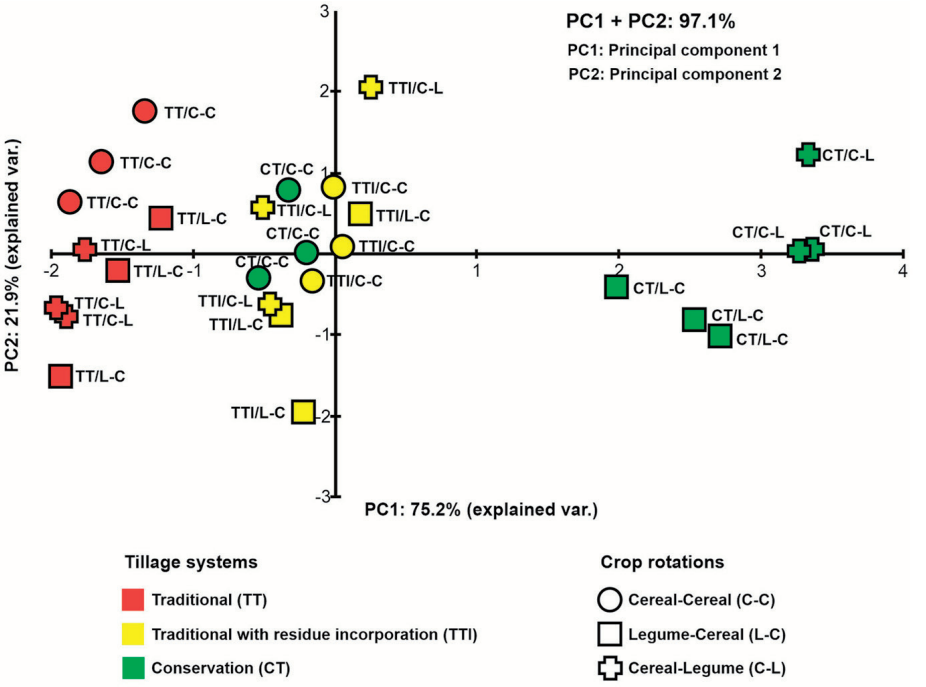


Table 8. Characteristic vectors of the principal components for the microbial populations evaluated in the Tillage systems×Soil depths (0-5, 5-15, and 15-30 cm) interactions over a period of six crop cycles.

Variable (microbial populations)	Characteristic vectors	
	PC1	PC2
Bacteria	0.441481	-0.609447
Actinomycetes	0.351374	0.770668
Fungi	0.569648	0.133271
Total	0.597605	-0.129937
Characteristic value	2.71381084	0.96927093
Explained variance, %	67.8	24.2

PC1: Principal component 1; PC2: Principal component 2.

The results of the principal component analysis of the treatments evaluated in the Tillage×Depth interaction are shown in Figure 2. In PC1, the largest populations of fungi and bacteria were present under CT/0-5 cm and the lowest in TT/15-30 cm. Bu et al. (2020) observed increases in bacterial populations when there were crop residues because in CT, soil movement was zero and the cover with crop residues was left as mulch on the surface. C and N, as well as moisture, were more abundant on the soil surface, which results in an increase in the levels of microbial activity (Zuber and Villamil, 2016; Somenahally et al., 2018). The central part of the graph shows the TTI treatment with all the profiles. In turn, the population of actinomycetes remained constant in and similar between TT and TTI, but there were variations in the CT, where actinomycetes decreased as the depth increased (CT/15-30). The results agree with those reported by Hartmann et al. (2009), who noted that soil profiles are influenced by the environment and by multiple edaphic factors that change with depth. One of the most pronounced changes throughout the profiles was the almost exponential decrease in microbial biomass, a pattern that paralleled the decrease in C, an abundant surface component in the cover residues.

Table 9 shows the principal component analysis of the Tillage×Crop cycle interaction. The most important variables for principal component 1 (PC1) were fungi, bacteria, and total microbes, and for PC2, it was actinomycetes alone. Both principal components together explained 80.6% of the total variation generated.

Figure 2. Principal components analysis of microbial populations evaluated in soil under different tillage systems and three soil depths over period of six crop cycles.

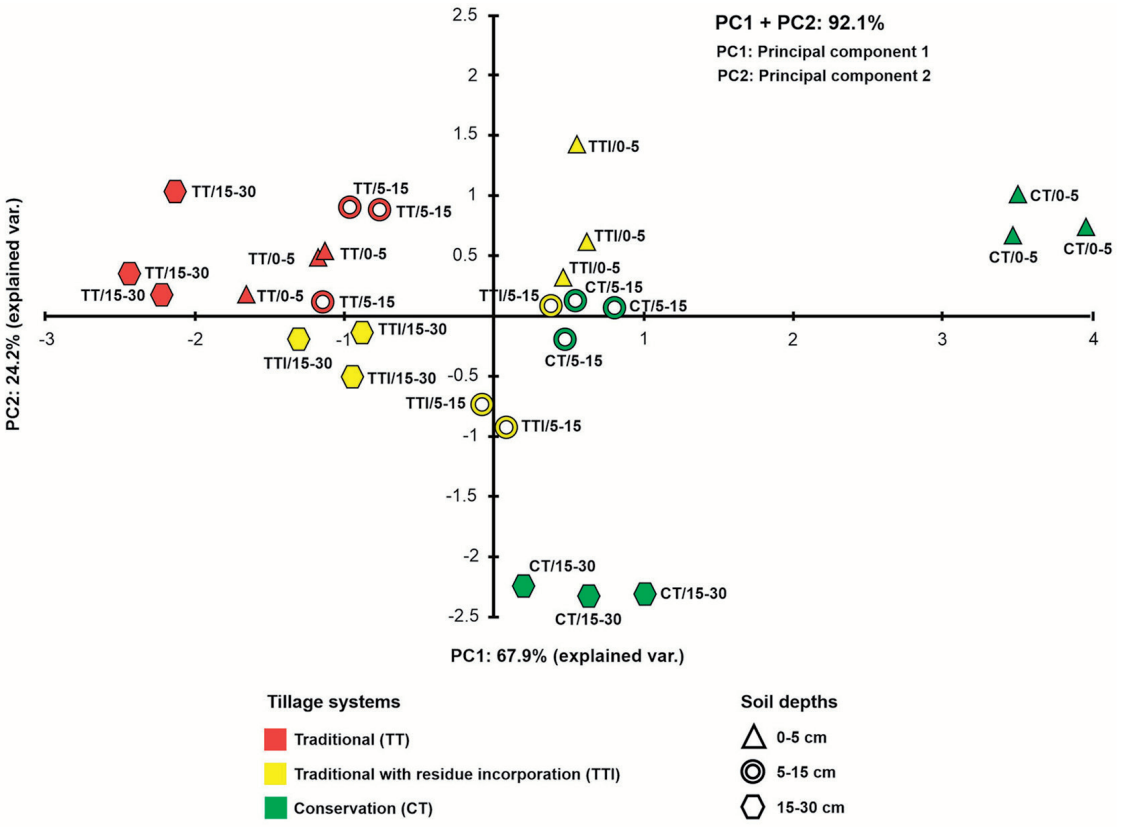


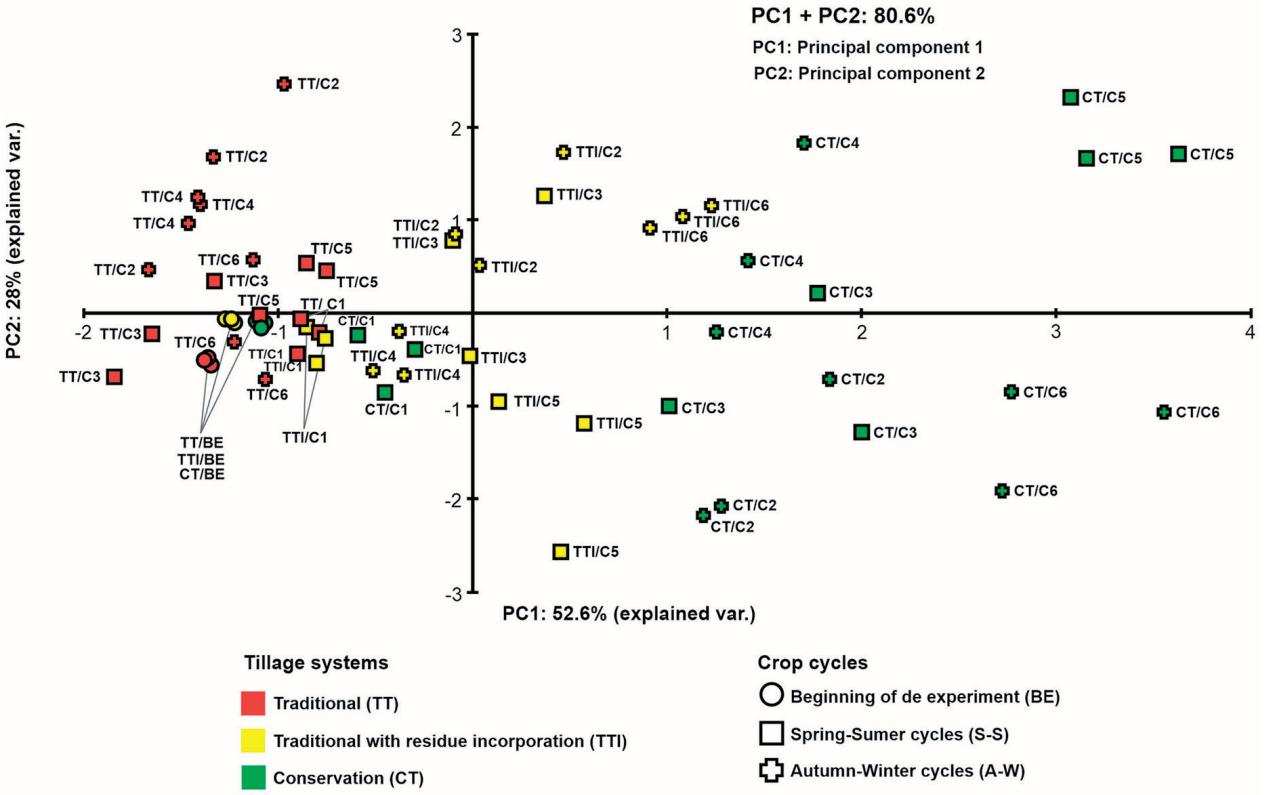
Table 9. Characteristic vectors of the principal components for the microbial populations evaluated in the Tillage systems×Crop cycle interaction in the 0-30 cm soil profile.

Variable (microbial populations)	Characteristic vectors	
	PC1	PC2
Bacteria	0.321243	-0.694601
Actinomycetes	0.228549	0.673349
Fungi	0.617264	0.229724
Total	0.680848	-0.10657
Characteristic value	2.1057415	1.1199067
Explained variance, %	52.6	28.0

PC1: Principal component 1; PC2: Principal component 2.

Figure 3 shows the behavior of the microbial populations in the different tillage systems over time. In CT, the fungal and bacterial populations increased, especially in cycles 5 (S-S) and 6 (A-W). In turn, differences were found in the actinomycete populations, probably related to the temperature conditions and rainfall distributions of each cycle. Martín and Rivera (2000) noted that a stable cover helps increase the organic matter content and improves the soil structure, protecting it from the impact of rainfall and solar radiation, favoring the thriving of soil microbiota. The tillage system that allowed the next-greatest concentration of microorganisms was TTI, and the maximum values were obtained in TTI/C5 and TTI/C6. In turn, under TT, the fungal and bacterial populations stayed low, while the actinomycetes increased in the A-W cycles (C2, C4, and C6). The type of tillage influences the transformation of nutrients (Cheng et al., 2017). Residue mantles or incorporation of residues can influence the stability of the soil microclimate (Blanco-Canqui and Ruis, 2018), the distribution and decomposition of raw organic matter, and the organic matter content (Li et al., 2018; Somenahally et al., 2018).

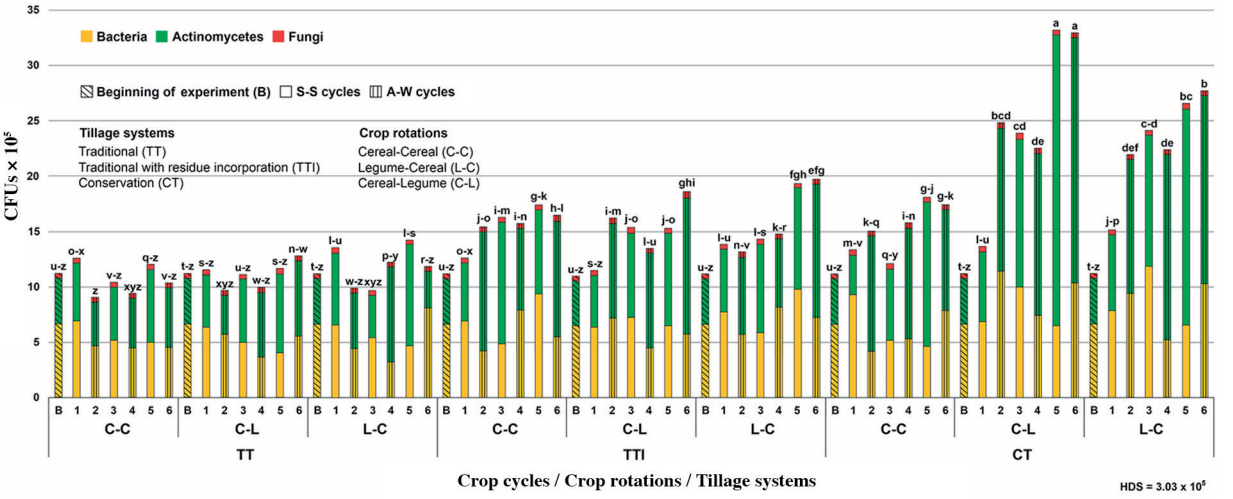
Figure 3. Principal components analysis of microbial populations evaluated in soil under different tillage systems and six crop cycles in the 0-30 cm soil profile.



The results on the total microbial populations in the Tillage×Rotation×Crop cycle interaction are presented in Figure 4. The largest populations were observed under CT/C-L in cycles 5 and 6. The TT showed different trends according to the rotation used. A decrease in microbial populations was observed in C-C, and the lowest values occurred in the second crop cycle, but there were nonsignificant differences. In the C-L rotation, the increase was 13.7%, and in L-C it was 5.4%. Under TTI, the populations increased 47.4% with C-C, 68.8% with C-L, and 76.2% with L-C. Under CT, the increases were greater: 55.4% with C-C, 193.0% with C-L, and 144% with L-C. A greater increase in microbial populations has been observed in tillage systems where residues were incorporated, which improved the soil quality (Zuber and Villamil, 2016; Somenahally et al., 2018; Bu et al., 2020). The average climatic conditions of temperature and rainfall of the S-S and A-W cycles held steady during the experiment; however, there were variations in the rainfall distribution, which affects the soil microclimate, as indicated by Blanco-Canqui and Ruis (2018).

Regarding the conformation of the total populations, the greatest variation was observed for actinomycetes, followed by bacteria. The fungal population was more constant in all tillage systems and crop rotations. The largest populations of actinomycetes were present under CT; in the last cycles, they represented more than 50% of the total microbial population. Bhatti et al. (2017) noted that actinomycetes are the main group of soil microorganisms that play a major role in recycling of organic matters in environment by production of hydrolytic enzymes, in addition to improving the availability of nutrients and minerals, synthesizing plant growth regulators, and, in particular, inhibiting phytopathogens. Agricultural practices that increase their populations will help improve the soil and build a sustainable agricultural system.

Figure 4. Changes in soil microbial communities in soil under different tillage systems and crop rotation over period of six crop cycles in the 0-30 cm soil profile.



Means followed by different letter in the bars are significantly different according to the HSD test ($p \leq 0.05$).
S-S: Spring-summer; A-W: autumn-winter; CFUs: colony forming units.

CONCLUSIONS

The tillage system and crop rotation directly affected the patterns of microbial populations in the soil. In the traditional tillage/cereal-cereal (TT/C-C) treatment (regional control), the microbial populations decreased, and over time, the microbiological degradation of the soil may become irreparable. When comparing the types of tillage, conservation tillage (CT) showed the greatest increases in microbial populations. Likewise, when there was cereal-legume (C-L) or legume-cereal (L-C) rotation, traditional tillage with residue incorporation (TTI) contributed to significant increases in microbial populations, equaling those under CT/C-C and CT/C-L. Rotations with different species had different effects, and C-L contributed to increasing the populations in all three types of tillage. Regardless of the treatment, fungal populations remained stable throughout the experiment, and those of actinomycetes increased considerably under TTI and CT. Regarding the depth of the soil profile, in all treatments the concentration of microorganisms decreased with depth. The study of microbial behaviors in the soil is essential to identifying agronomic strategies to prevent or correct their degradation.

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