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Effects of land uses and soil types on microbial activity and community structure**

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A b s t r a c t. This study was conducted in order to understand the effects of land use and soil types on microbial activity and community structure. Soil samples were collected from four different soil types (Solonetz, Solonchak, Chernozem and Gleysol) being used under different land use practices (arable, pasture and meadow). The soil chemical properties, moisture content, microbiological activity and community size were investigated. The principal component analysis results showed that different land uses and soil types are clearly separated based on the chemical properties of the soil. The canonical correspondence analysis results revealed that more than 78% of variation in the microbiological properties of the samples could be explained by environmental factors. Significant biological differences were observed among the different land use practices and soil types, and also soil cultivation affected the different groups of soil microbes. Sampling sites were separated into two main clusters (Bray-Curtis) based on certain microbiological properties, salt-affected and non-salt-affected soils. The soil types were the main driving factor, with high soil taxonomic distances, however, low taxonomic distances indicated that land use had more pronounced effects on soil microbiological properties.

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K e y w o r d s: soil properties, PLFA, salt-affected soils, enzyme activity, soil taxonomic distances

INTRODUCTION

There is a close interaction between land use and soil properties as land use practices affect the soil quality, soil functions and ecological processes due to modifications in the physical, chemical and biological properties of the soils (Pouyat *et al.*, 1995; Bending *et al.*, 2002; Balota *et al.*, 2003; Bardgett *et al.*, 2014; Wu *et al.*, 2020; Gangwar *et al.*, 2021). Several management strategies have been implemented over the years which are different from the inherited sustainable use of the land. These management practices have led to the overuse/utilization or abandonment of land (Pinto-Correia and Mascarenhas, 1999). Non-sustainable land use practices may even result in severe soil degradation processes (Costa *et al.*, 2013).

Microbial communities perform various functions that are essential for the maintenance of soil multi-functionality (Winding, 2004). Soil microbial communities play a fundamental role in supporting plant/crop growth by regulating nutrient cycling, organic matter decomposition and ecosystem processes that are important for the growth and maintenance of plants (Lehman *et al.*, 2015a; Ren *et al.*, 2018; Fanin *et al.*, 2019). Soil environmental

conditions such as physicochemical factors govern the composition and diversity of these microbial communities (Bass Becking, 1934), which are particularly important to sustainable agriculture (Bender *et al*., 2016; Lakshmanan *et al*., 2014; Lehman *et al*., 2015b). Thus, developing our understanding of microbial communities and how various management practices (*i.e.*, different land use practices) impact these communities and their diversity is of the utmost importance.

Assessing the microbial community structure in soils was difficult until the development of phospholipid fatty acid (PLFA) analysis (Bobbie and White, 1980) which is presently an increasingly popular method which may contribute to our understanding of ecosystem function and sustainable land management (Veum *et al*., 2019). PLFA analysis has been used in several studies which reported the impact of different land use practices on microbial community structure (Krashevska *et al*., 2015; Yuan *et al*., 2015; Guo *et al*., 2016; Ahmed *et al*., 2019; Gangwar *et al*., 2019; Liu *et al*., 2020). Ahmed *et al*. (2019) and Rampazzo *et al*. (1999) showed that soil microbial properties and enzyme activities were significantly different among the various land-use types. Moreover, Yuan *et al*. (2015) investigated the effects of land use practices on the composition of the soil microbial community by analysing soil PLFA and found that the soil microbial community structure varied to a significant extent. A meta-analysis of various land use changes revealed that microbial attributes and their determinants were particularly affected by the types of land use changes. A decline in the soil microbial community in anthropogenic cases was driven by organic carbon, total nitrogen and the C:N ratio, while in the case of natural changes the following factors played key roles; total nitrogen, phosphorus and the C:N ratio (Chen *et al*., 2022). According to Moran-Rodas *et al*. (2022), who investigated two soil types under different land uses, the highest impact factor on the soil microbial community was particulate soil organic matter content.

In this study, we sought to assess the potential effects of different land uses and soil types on microbial communities and activities and also, the main soil chemical properties driving the microbial parameters were investigated. Our hypothesis was that the chemical and physical properties of the soil have greater influences on microbiological activities and communities, modified by the different land use types.

MATERIALS AND METHODS

Soil samples were collected from Nádudvar (Hajdu-Bihar County), Apaj (Pest County) and Szappanszék (Bács-Kiskun county) in Hungary (Fig. 1). Table 1 presents the soil reference groups and land use types of the studied sites.

The cultivated arable site (NSnA and NChA) was ploughed to a depth of 30 cm and 400 kg ha^{-1} NPK (18:7:7) fertilizer was applied to the maize crop. The non-ploughed meadow site (NSnM) has not been cultivated for more than 30 years, while the Apaj site (AScP) was grazed by sheep and hence this site received grazed animal droppings and both (NSnM and AScP) sites had a continuous covering of grassy vegetation. Szappanszék (SGlP) has been a protected area since 1975 and belongs to the Kiskunság National Park, with a covering of grassy vegetation and extensive ox grazing.

One central soil profile at each site was described (FAO, 2006) and classified according to the IUSS Working Group WRB (2014 - updated 2015) in order to characterize the pedological conditions. The soil samples were collected from the surface soil (0-15 cm) in the summer of 2017. For the collection of soil samples, two plots of 100 m^2 were selected from each site within a radius of 60 m from the central profile. Ten soil subsamples were randomly collected and combined to make a well-mixed composite sample from each plot. All of the vegetation and litter from the soil surface was removed before sampling. Collected soil samples were placed in plastic bags and transported to the laboratory in a cooling box. The samples were sieved through a 2 mm sieve to obtain a well-homogenized sample. For chemical analysis, the sieved soils were air dried and stored at room temperature (28°C). For microbiological analysis, the samples were stored at –20°C. Before microbiological analysis, the soils were stored at 4°C for one night.

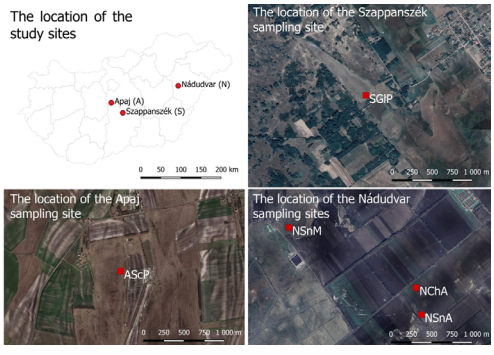

Fig. 1. Location of the sampling sites.

Table 1. Soil reference groups and land use types of the studied sites

Sampling site	Soil reference group (RSG)	Land use type	Abbreviation used
Nádudvar (N)	Solonetz (Sn)	Arable (A) land	NSnA1; NSnA2
	Chernozem (Ch)	Arable (A) land	NChA1; NChA2
	Solonetz (Sn)	Meadow (M) land	NSnM1; NSnM2
Apaj (A)	Solonchak (Sc)	Pasture (P) land	AScP1; AScP2
Szappanszék (S)	Gleysol (Gl)	Pasture (P) land	SGlP1; SGlP2

Three replicates were used for each measured parameter. Soil pH and electrical conductivity (EC) were measured in a soil-water suspension (1:2.5) (Buzás, 1988). The soil organic carbon (OC) was determined by Walkley and Black (1934) method. Humic material (E4/E6) was determined using a method developed by Page *et al*. (1982). Plant available AL-(ammonium lactate) P_2O_5, AL-K_2O and plant available nutrients ($avNa^+$, $avCa^{2+}$ and $avMg^{2+}$) were extracted according to Egner *et al*. (1960). The soil moisture content was determined using a gravimetric method (Buzás, 1993).

Soil profile samples were collected for soil classification. Samples from different horizons were sieved (<2 mm), air dried and stored for chemical and physical analysis. The chemical analyses of OC, EC and pH were determined using the above-mentioned methods whereas exchangeable basic cations (S value) were determined based on the modified Mehlich method (Mehlich, 1953) while the exchangeable sodium percentage (ESP %) was calculated using the following: exchangeable sodium ($exNa^+$)/(sum of $exNa^+$, $exCa^{2+}$, $exMg^+$ and exK^+) *100. A pipette method (Buzás, 1993) was used to determine particle-size distribution.

Soil microbial biomass carbon (MBC) was estimated using the chloroform fumigation-extraction method (Brookes *et al*., 1985; Vance *et al*., 1987). Microbial activity or basal soil respiration (BSR) represents the feedback of microbes for organic substrates. The alkali absorption method was used to measure BSR. It was measured in terms of the CO_2 which evolved at the optimum water content (60% field capacity) of the soil (Carter, 1993; Cheng *et al*., 2013). The activity of the phosphatase enzyme was measured as described by Tabatabai and Bremner (1969). This involves calorimetric estimation of the p-nitrophenol released by phosphatase activity. Dehydrogenase activity (DHA) was determined by the transformation of 2,3,5-triphenyltetrazolium chloride (TTC) to 1,2,5- triphenylformazan (TPF) (Casida *et al*., 1964).

PLFA indicator molecules were determined from soil samples based on a modified method of White *et al*. (1979). The prepared samples were stored at –20ºC until an analysis was performed using a gas chromatograph-mass spectrometer system (GC 6890N with MS 5975, Agilent, Santa Clara, CA, USA) with a 100 m Supelco SP-2560 column, in selected ion mode and scan mode as well (50-350 amu). For PLFA identification methyl nonadecaonate was used as an internal standard. The unbranched, saturated PLFAs such as C14:0, C15:0, C16:0 and C18:0 were used as general bacterial markers. Branched, saturated PLFAs iC15:0, aC15:0, iC16:0, iC17:0 and aC17:0 were used to indicate Gram-positive bacteria. Gram-negative bacteria were characterized using monoenoic and cyclopropane with unsaturated C18:1n9c and cyC19:0 PLFAs (Gude *et al*., 2012). 10MeC16:0 and 10MeC17:0 PLFAs were used to quantify Actinobacteria (Dong *et al*., 2014) and C16:1n5c for arbuscular mycorrhiza fungi (AMF) (Marshall *et al*., 2011). Polyunsaturated C18:2n6c, C18:3n6c and c18:3n3 were used as Fungi markers (Nakatani *et al*., 2012). The total PLFA content was calculated as the sum of the above-mentioned PLFAs. Moreover, the ratios of Gram-negative to Gram-positive Bacteria, Fungi to General Bacteria and Actinobacteria to General Bacteria were calculated.

The analyses of variance (ANOVA) of the data from different sites were computed using SPSS statistics v 23.0. The mean of the parameters of different sites were separated using a Tukey HSD post hoc test at the $p<0.05$ level. The chemical and physical properties of all of the composite samples were applied to calculate the principal component analyses (PCA). For clustering the sites based on their microbiological properties, Bray-Curtis analyses were carried out. A canonical correspondence analysis (CCA) was performed to predict the relationships between the microbiological properties and the environmental factors of the studied sites using PAST vs 3.

RESULTS

The lowest level of soil organic carbon (OC) was found in Szappanszék-Gleysols-SGlP1 (0.84%) and the highest in Nádudvar- Chernozems-NChA2 (7.82%) (Table 2). In the case of SG1P1 location a statistical difference was observed between the two sampling sites. Both Nádudvar-Solonetz soils (NSnA and NSnM) were not significantly different in terms of OC. The Apaj sites (AScP) represented statistically higher values than the Solonchak locations. The E4/E6 ratio ranged from 3.90 to 6.77. The E4/E6 values at SGlP1 and NChA2, and at SGlP2 and NSnM1 were not significantly different. However, the pH values were significantly different at the NChA and SGlP sites with the highest observed value occurring at SGlP2 (9.57) while it was lowest at NChA2 (6.13) whereas the EC ranged from 48.10 µS cm^{-1} (NChA2) to 392.87 µS cm^{-1} (AScP1). The values of P_2O_5, K_2O, Mg^+ and Ca^{2+} were found to be higher at both arable sites (NSnA and NChA) as compared to the meadow (NSnM) and pasture sites (AScP and SGlP). At NSnA and NSnM the values of K_2O were significantly different whereas at NChA and NSnM, the P_2O_5 values were significantly different. The highest Na^+ value was recorded at AScP1 (789.00 mg kg^{-1}) and the lowest at SGlP1 (172.33 mg kg^{-1}) which is not significantly different that found at NChA. In the case of soil moisture, AScP1 had the highest value (32.28 mg kg^{-1}) and SGlP1 had the lowest value (15.42 mg kg^{-1}). Soil moisture was found to be statistically different at all sites except NSnA2 and AScP1.

Overall, the soil BSR values were found to range from 1.59 µg CO_2 g^{-1} soil h^{-1} (NSnA1) to 5.42 µg CO_2 g^{-1} soil h^{-1} (AScP1) while the MBC values were found to be ranged from 74.86 µg C g^{-1} (NChA1) to 735.80 µg C g^{-1} (AScP1) (Table 3). The values of BSR were significantly different within the sampling sites of NSnA, NSnM and AScP whereas the MBC values were found to be significantly different at SGlP. The DHA values were found to be lowest at SGlP1 (4.95 µg formazan g^{-1} soil day^{-1}) and highest at AScP1 (520.64 µg formazan g^{-1} soil day^{-1}) and also, the

Table 2. Descriptive statistics ANOVA (mean ± standard deviation (SD)) of the soil moisture and chemical properties

Property	Unit	NSnA		NChA		NSnM		AScP		SGlP	
		1	2	1	2	1	2	1	2	1	2
OC	(%)	3.48± 0.12c	3.52± 0.20c	7.51± 0.26e	7.82± 0.04e	3.37± 0.06c	3.56± 0.05c	6.21± 0.81d	5.81± 0.19d	0.84± 0.05a	1.77± 0.08b
E4/E6		4.17± 0.06b	4.23± 0.06b	5.17± 0.06d	6.70± 0.20e	3.90± 0.00a	4.80± 0.00c	4.77± 0.06c	4.77± 0.06c	6.77± 0.06e	3.90± 0.00a
pH		6.97± 0.06c	7.00± 0.10c	6.77± 0.06b	6.13± 0.06a	8.47± 0.06e	8.47± 0.06e	8.10± 0.00d	8.10± 0.00d	9.00± 0.10f	9.57± 0.06g
EC	($\mu S\ cm^{-1}$)	136.4± 0.97bc	108.3± 6.21b	156.9± 11.29c	48.10± 1.91a	144.8± 6.12c	131.2± 4.05bc	392.9± 16.77e	262.2± 20.42d	51.20± 3.21a	141.7± 12.71c
P_2O_5	($mg\ kg^{-1}$)	420.3± 18.15d	410.0± 13.89d	545.0± 39.69e	650.0± 6.24f	283.7± 19.35c	162.0± 4.58b	55.80± 3.10a	48.77± 4.86a	31.50± 1.71a	42.90± 4.25a
K_2O	($mg\ kg^{-1}$)	377.7± 20.82d	471.7± 24.01e	303.7± 11.02c	298.3± 12.86c	352.0± 19.29d	187.7± 9.87b	203.7± 15.95b	196.0± 19.08b	43.43± 2.74a	83.43± 4.69a
Mg	($mg\ kg^{-1}$)	44.23± 10.66bc	42.33± 12.88abc	34.20± 9.65abc	37.20± 7.85abc	32.27± 8.69abc	31.43± 6.40abc	42.23± 8.42abc	49.57± 9.77c	18.90± 4.17a	19.53± 3.80ab
Ca	($mg\ kg^{-1}$)	1495± 385.5bc	1412± 297.4bc	1843± 434.3c	1859± 515.51c	899.0± 192.0ab	834.7± 260.3ab	668.3± 162.1ab	738.3± 187.5ab	145.7± 44.38a	116.8± 33.25a
Na	($mg\ kg^{-1}$)	350.0± 82.83ab	271.3± 45.83ab	237.7± 39.07a	227.0± 41.58a	498.0± 94.18bc	335.3± 78.87ab	789.0± 138.0d	692.0± 141.5cd	172.3± 45.00a	357.3± 64.69ab
Moisture	(%)	26.93± 2.90bc	31.27± 6.82c	18.71± 1.18ab	23.74± 1.70abc	23.85± 5.61abc	16.79± 1.83ab	32.28± 2.23c	22.27± 4.46abc	15.42± 3.72a	18.01± 3.78ab

Means in a column followed by the same letter are not significantly different at 5% level. Site abbreviations are used according to Table 1.

Table 3. Descriptive statistics ANOVA (mean ± standard deviation (SD)) of the soil microbiological properties

Property	Unit	NSnA		NChA		NSnM		AScP		SGlP	
		1	2	1	2	1	2	1	2	1	2
BSR	($\mu g\ CO_2\ g^{-1}\ soil\ h^{-1}$)	1.59± 0.30a	5.13± 0.88d	2.78± 0.23bc	2.78± 0.23bc	5.38± 0.05d	2.03± 0.08ab	5.42± 0.23d	3.09± 0.12c	2.96± 0.14bc	3.07± 0.14c
MBC	($\mu g\ C\ g^{-1}$)	197.5± 26.59b	182.1± 27.10ab	74.86± 0.04a	182.3± 26.57ab	493.1± 74.20d	414.4± 5.63cd	735.8± 24.41e	717.2± 58.05e	159.0± 24.35ab	320.3± 47.37c
DHA	(μg formazan g^{-1} soil day^{-1})	39.42± 0.09b	82.36± 0.26d	83.47± 0.98d	76.37± 0.21c	282.4± 0.67g	152.1± 1.23f	520.6± 1.69i	370.0± 0.51h	4.95± 0.03a	102.9± 1.03e
Phosphatase	($\mu mol\ PNP\ g^{-1}\ soil\ h^{-1}$)	0.09± 0.000a	0.09± 0.000a	0.13± 0.001d	0.12± 0.001c	0.27± 0.001f	0.24± 0.001e	0.83± 0.006h	0.73± 0.002g	0.13± 0.000d	0.10± 0.001b

Means in a column followed by the same letter are not significantly different at 5% level. Site abbreviations are used according to Table 1.

DHA values were significantly different at all sampling sites except for NSnA2 and NChA1. Whereas the lowest phosphatase activity was observed at both plots of NSnA (0.09 μmol PNP g^{-1} soil h^{-1}) the highest value was determined at AScP1 (0.83 μmol PNP g^{-1} soil h^{-1}). The values of phosphatase activity were not found to be significantly different for NChA1 and SGlP1 while it was determined to be significantly different within each sampling site (except NSnA).

The interaction between land use and the chemical properties of the soil were investigated using principal component analysis (PCA, Fig. 2). Component 1 and component 2 explained 71.69 and 21.50% of the total variance, respectively. The effect of land use was reflected in component 1 with positive values for arable land and meadow land in the centre, and negative ones for pasture land. The first component was determined positively by P_2O_5 and Ca while the second component was contributed positively by EC and Na. Specifically, the arable lands (NSnA and NChA) had higher amounts of plant available P_2O_5 and Ca, while the pasture land (AScP) could be characterized by a high EC and Na content. The different soil types and land uses could be separated clearly.

The highest value of general bacterial PLFAs was measured at the NSnM2 site (14.64 nmol g^{-1} soil) while this value was slightly lower at AScP1 (13.48 nmol g^{-1} soil) and AScP2 (14.05 nmol g^{-1} soil) sites. The general bacterial PLFA

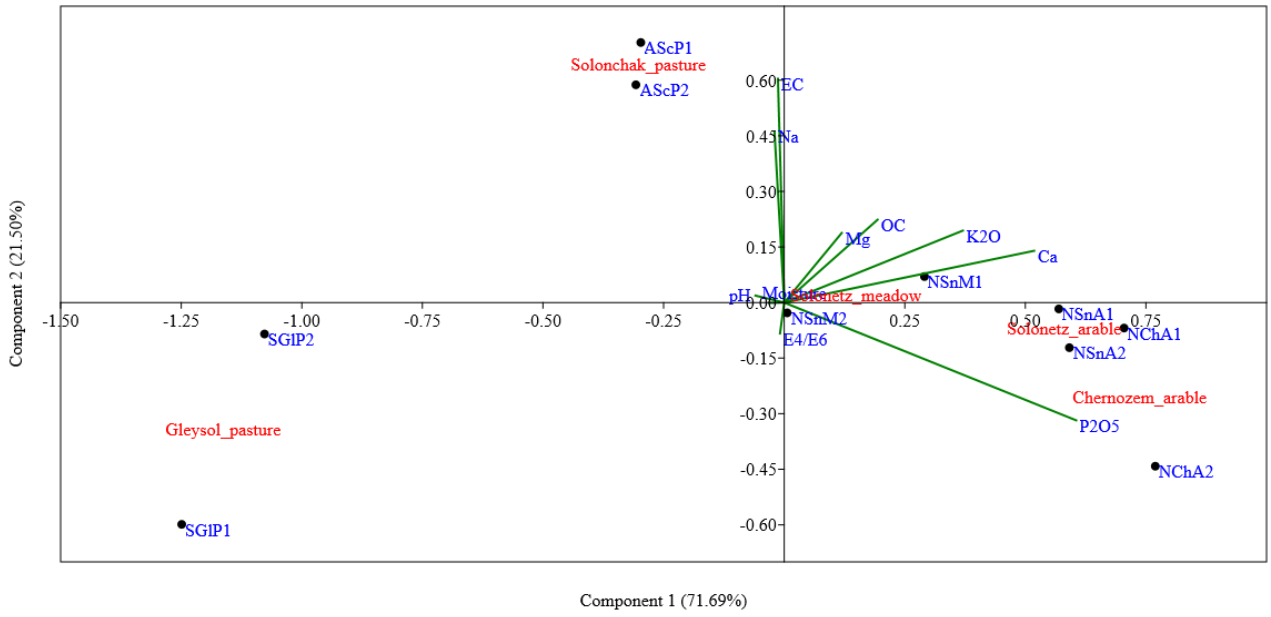

Fig. 2. Results of the principal component analysis based on the chemical properties and moisture content.

values indicated the existence of a smaller bacterial community at the sites of NSnM1 (10.83 nmol g^{-1} soil) and SGlP2 (7.205 nmol g^{-1} soil), followed by the arable sites (NSnA1, NSnA2, NChA1, NChA2) and site SGlP2 with a range of 4.012-5.570 nmol g^{-1} of dry soil (Table 4).

In the case of Gram-positive bacterial PLFAs, the highest value was also measured at the NSnM2 site (16.69 nmol g^{-1} soil) followed by the AScP2 (16.07 nmol g^{-1} soil), AScP1 (16.28 nmol g^{-1} soil) and NSnM1 (13.70 nmol g^{-1} soil) sites. Intermediate values of 7.43 nmol g^{-1} dry soil PLFAs was measured at the SGlP2 site, followed by 6.187 nmol g^{-1} soil and 5.324 nmol g^{-1} dry soil PLFAs that were measured at the NSnA2 and NSnA1 sites, respectively. The lowest values were found at the NChA2 (4.893 nmol g^{-1} soil) and NChA1 (5.088 nmol g^{-1} soil) sites.

The range of measured Gram-negative bacterial PLFAs revealed the existence of a smaller Gram-negative bacterial community in soils than Gram-positive ones. The results indicated that the largest Gram-negative bacterial community occurred at the AScP2 (4.448 nmol g^{-1} soil) and AScP1 (4.358 nmol g^{-1} soil) sites. Intermediate results were measured at the NSnM2 (3.909 nmol g^{-1} soil) and NSnM1 (3.059 nmol g^{-1} soil) sites for which the mean values were only the 79.09% of AScP sites, compared to the 93.93% measured in the case of Gram-positive PLFA

Table 4. Descriptive statistics ANOVA (mean ± standard deviation (SD)) of the soil PLFA properties (nmol PLFA g^{-1} soil)

Parameter	NSnA1	NSnA2	NChA1	NChA2	NSnM1	NSnM2	AScP1	AScP2	SGlP1	SGlP2
General bacteria	4.721± 0.163b	4.725± 0.075b	4.012± 0.013a	4.035± 0.162a	10.83± 0.092e	14.64± 0.093h	13.48± 0.009f	14.05± 0.033g	5.570± 0.041c	7.205± 0.041d
Gram-positive	5.328± 0.186b	6.187± 0.137c	5.088± 0.200ab	4.893± 0.158a	13.70± 0.067e	16.70± 0.210g	16.28± 0.042f	16.07± 0.136f	4.722± 0.068a	7.434± 0.036d
Gram-negative	1.571± 0.104bc	1.272± 0.103a	1.683± 0.066c	1.511± 0.074b	3.059± 0.107e	3.909± 0.025f	4.358± 0.054g	4.448± 0.004g	1.276± 0.014a	2.645± 0.017d
Actino-bacteria	1.942± 0.046b	2.101± 0.029bc	2.524± 0.103c	2.340± 0.101bc	3.495± 0.051d	4.410± 0.049e	9.021± 0.258g	7.798± 0.373f	1.346± 0.031a	2.480± 0.015c
AMF	0.177± 0.012b	0.171± 0.002b	0.171± 0.008b	0.159± 0.008ab	0.402± 0.002d	0.494± 0.013e	0.591± 0.011f	0.648± 0.003g	0.145± 0.004a	0.275± 0.014
Fungi	0.507± 0.011d	0.361± 0.002b	0.251± 0.017a	0.398± 0.022c	1.478± 0.003h	1.507± 0.006h	1.421± 0.001g	2.090± 0.005i	0.603± 0.005e	1.160± 0.007f
Total PLFA	14.25± 0.514bc	14.82± 0.274c	13.73± 0.447ab	13.33± 0.149a	32.96± 0.134e	41.66± 0.322f	45.16± 0.276g	45.10± 0.424g	13.66± 0.148ab	21.20± 0.077d

Means in a column followed by the same letter are not significantly different at 5% level. Site abbreviations are used according to Table 1.

indicators. The SGlP2 site (2.645 nmol g^{-1} soil) also had a higher PLFA content than the arable and SGlP1 sites (1.272-1.683 nmol g^{-1} dry soil).

The largest Actinobacteria community was found in the AScP1 (9.021 nmol g^{-1} soil) and AScP2 (7.798 nmol g^{-1} soil) sites while the corresponding PLFA concentrations were found to be much lower at the NSnM2 (4.410 nmol g^{-1} soil) and NSnM1 (3.495 nmol g^{-1} soil) sites followed by the arable and Gleysol pasture sites (2.254-1.346 nmol g^{-1} soil).

The volume of the AMF community was more similar in the case of the AScP and NSnM sites as compared to the Actinobacteria results. In detail, the highest values were measured at the NSnP2 (0.648 nmol g^{-1} soil) and NSnP1 (0.591 nmol g^{-1} soil) sites followed by the results of the NSnM2 (0.494 nmol g^{-1} soil) and NSnM1 (0.402 nmol g^{-1} soil) sites. At the remaining arable and Gleysol pasture sites the PLFA results indicated smaller AMF communities with a range of 0.145-0.176 nmol g^{-1} dry soil values.

The fungal communities were larger at the AScP2 (2.090 nmol g^{-1} soil), NSnM2 (1.507 nmol g^{-1} soil), NSnM1 (1.478 nmol g^{-1} soil), AScP1 (1.421 nmol g^{-1} soil) and SGlP2 (1.160 nmol g^{-1} soil) sites than at the SGlP1 (0.603 nmol g^{-1} soil), NSnA1 (0.507 nmol g^{-1} soil), NChA2 (0.398 nmol g^{-1} soil), NSnA2 (0.361 nmol g^{-1} soil) and NChA1 (0.251 nmol g^{-1} soil) sites.

Summarizing, the results of different microbial groups, the largest communities were found at the AScP1 (45.16 nmol g^{-1} soil) and AScP2 (45.10 nmol g^{-1} soil) sites followed by the NSnM2 (41.66 nmol g^{-1} soil) and NSnM1 (32.96 nmol g^{-1} soil) sites. While the total PLFA results were similar in the case of the AScP sites, the two sites of the NSnM land were different from each other. The greatest difference between the PLFA communities was found in the SGlP sites where 21.20 nmol g^{-1} dry soil PLFA concentration was measured in the SGlP2 site while this value was only 13.66 nmol g^{-1} soil in the SGlP1 site. In the arable sites the total PLFA content was much lower than in the AScP and NSnM sites, it ranged from 13.33 to 14.82 nmol g^{-1} soil with higher values for the NSnA sites than the NChA sites.

The ratios of the PLFA groups indicate the biological properties of soils in different sites as a function of the environmental circumstances (Table 5). The lowest ratios of G-negative/G-positive bacteria were found at the NSnM1 (0.223), NSnM2 (0.234) and NSnA2 (0.206) sites followed by the AScP1 (0.268), SGlP1 (0.270) and AScP2 (0.277) sites. Higher results were measured at the arable NSnA1 (0.295), NChA2 (0.309) and NChA1 (0.331), as well as the pasture SGlP2 (0.356) sites.

The means of the fungi/general bacterial PLFAs were also separated into different groups. A considerably low ratio was found at the NChA1 (0.062) site, which was followed by the NSnA2 site (0.077). The third group contained the remaining arable sites NChA2 (0.099), NSnA1 (0.107), the meadow NSnM2 (0.103) site and, the pasture AScP1 (0.105) and SGlP1 (0.108) sites. The highest ratios were calculated for the NSnM1 (0.137), AScP2 (0.149) and SGlP2 (0.161) sites.

The Actinobacteria to general bacteria ratios varied substantially according to the land use practices. The ratio was low at two of the land types studied: the lowest ratio was calculated for the SGlP1 (0.242) site followed by the NSnM2 (0.301), NSnM1 (0.323) and SGlP2 (0.344) sites. The Solonetz arable sites (NSnA1 and NSnA2) had similar ratios (0.411 and 0.445, respectively). The highest ratios were recorded for Solonchak pasture and the Chernozem arable sites from 0.555 to 0.669.

Analysing the microbiological properties of the studied sites revealed some similarities and dissimilarities. For a deeper analysis of this question, a Bray-Curtis distance analysis was carried out with all of the measured microbiological properties. This revealed that the sampling sites could be separated into two main clusters based on the microbiological properties: salt-affected and non-salt-affected soils (Fig 3). The Solonetz arable (NSnA), Solonchak pasture (AScP) and Chernozem arable (NChA) sites formed different clusters. The NSnM2 site was separated from the NSnM1, which is closer to the Apaj pasture sites. The Chernozem arable (NChA) site was also grouped with the Gleysol pasture site, this is presumably due to the lower moisture and Na^+ content.

One sampling site from the Gleysol pasture (SGlP1) was separated from the rest of the cluster which may indicate significant differences in microbiological properties as compared to other measured sampling sites. This indicates the highly heterogeneous microbial properties within the sampling site as well.

Table 5. Descriptive statistics of ANOVA (mean ± standard deviation (SD)) of the ratios of PLFA groups

Parameter	NSnA1	NSnA2	NChA1	NChA2	NSnM1	NSnM2	AScP1	AScP2	SGlP1	SGlP2
Gram-/Gram+	0.295± 0.010bc	0.206± 0.017a	0.331± 0.005de	0.309± 0.025cd	0.223± 0.001a	0.234± 0.003a	0.268± 0.004b	0.277± 0.002b	0.270± 0.002b	0.356± 0.003e
Fungi/bacteria	0.107± 0.002c	0.077± 0.001b	0.062± 0.004a	0.099± 0.010c	0.137± 0.001d	0.103± 0.000c	0.105± 0.000c	0.149± 0.001e	0.108± 0.001c	0.161± 0.002f
Actinobacteria/ General bacteria	0.411± 0.006d	0.445± 0.001d	0.629± 0.020f	0.580± 0.019e	0.323± 0.007bc	0.301± 0.001b	0.669± 0.019f	0.555± 0.0261e	0.242± 0.004a	0.344± 0.000c

Means in a column followed by the same letter are not significantly different at 5% level. Site abbreviations are used according to Table 1.

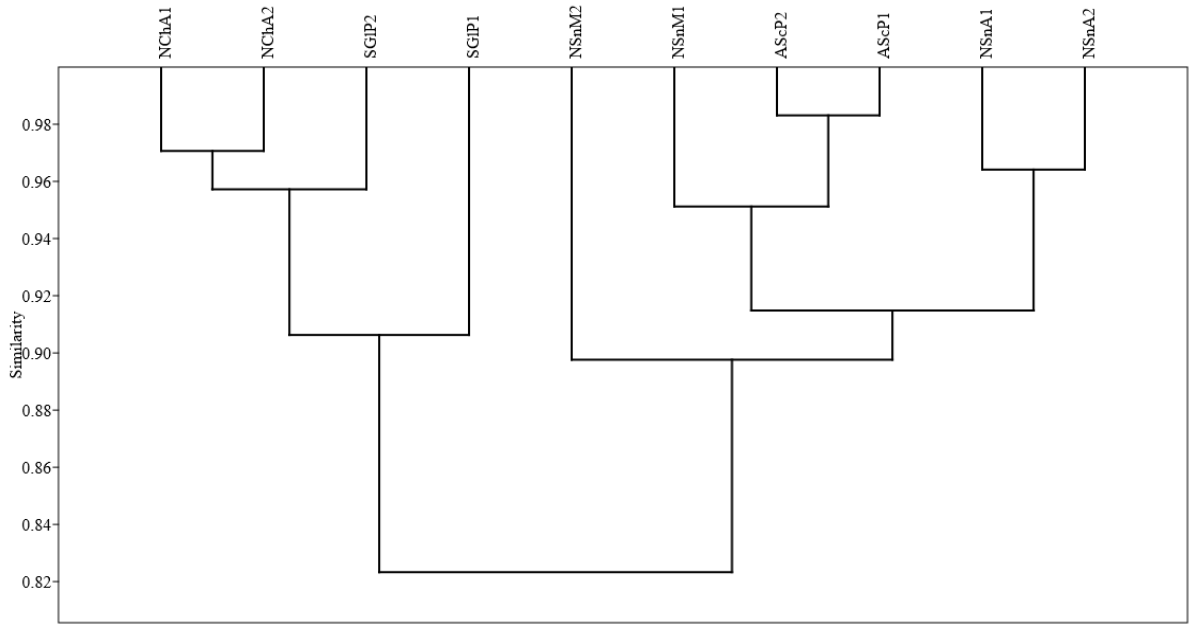

Fig. 3. Cluster analysis (Bray-Curtis) of the samples based on the investigated soil biological properties.

In the case of arable sites (NChA, NSnA) the inherited soil properties appearing in the soil classification, have a greater influence on the soil community structure than land use. Contrary, in the case of salt affected soils, the land use was the major driving factor which separated the two sites, as the Nádudvar Solonetz meadow (NSnM) sites are closer to the Apaj Solonchak pasture (AScP) sites in terms of their microbiological properties. The similarity between the Chernozem and Gleysol sites originated from their non-salic, or sodic properties.

CCA was used to determine the main environmental parameters affecting microbiological properties including PLFA (Fig 4.), the first two axes described 47.63 and 30.95% of variance. On Axis 1 the moisture content was the main factor which positively affected soil respiration, microbial biomass carbon, DHA and phosphatase activity while general bacteria, Gram-positive bacteria, Actinobacteria, AMF, Gram-negative bacteria and Fungi were all influenced negatively. Whereas on Axis 2, OC, EC, Mg and Na were the main environmental factors which positively affected DHA, phosphatase activity, Actinobacteria, AMF and

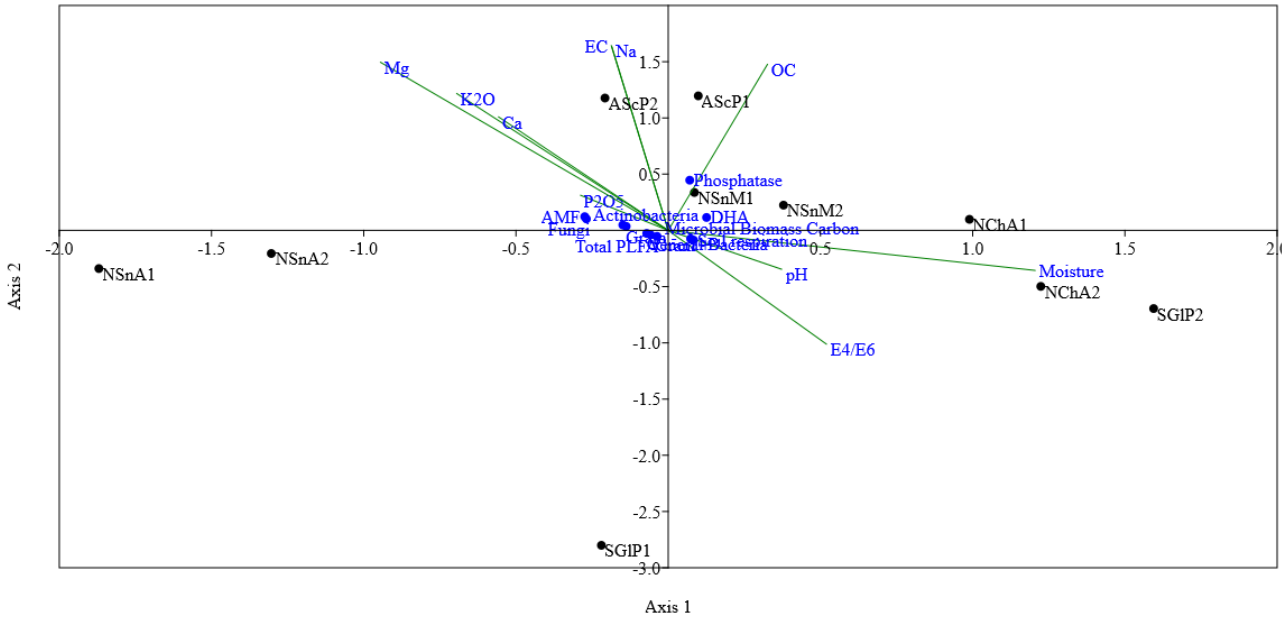

Fig. 4. Canonical Correspondence Analysis of the sites.

Fungi while soil respiration, MBC and Total PLFA were negatively influenced. Sampling sites with different soil types and land use practices were distributed near the origin but both arable sites (NSnA and NChA) were separated along the first axis together with the SGlP2 site while the AScP sites were separated along Axis 2 together with the SGlP1 site. The loadings of the NSnM sites were $p<0.05$.

DISCUSSION

Land use types may have positive and/or negative effects on the physical, chemical and biological properties of the soil (Bossio *et al*., 2005; Steenwerth *et al*., 2003; Xu *et al*., 2017). Organic matter input, a favourable soil pH, neutral or slightly alkaline, and the accumulation of nutrients improved the biological status of the soil (Kooch *et al*., 2018; Negasa *et al*., 2020). Furthermore, the soil microbial properties and enzyme activities were influenced by the soil organic matter content and affected by the land use type or soil management practices (Meena and Rao, 2021).

Chernozems are the most fertile soils in Hungary, covered with ancient grassy vegetation, on loessy soil parent material with dominant biological processes and high soil organic matter content providing perfect medium for successful plant production. As a result, these soils have been under cultivation for hundreds of years (Szűcs, 1959).

The measured soil chemical properties in the case of the salt-affected soils correspond to the results of Szabolcs and Jassó (1959). The low soil OC content (Table 2) and variability in the case of the Szappanszék Gleysols may be due to various soil forming factors as that area used to be a salt-affected lake, and because of global climate change the groundwater table has decreased, and as a consequence the area has turned into grassland (Tóth *et al*., 2015; Wiesmeier *et al*., 2014). The relatively high organic carbon content in the case of Solonchak was due to continuous plant coverage and the rarity of any disturbance (Tejada *et al*., 2006; Ayoubi *et al.,* 2020). 15 years of cultivation decreased the organic matter content of the ploughed layer by 12-22% in the Solonetz pasture in Hungary (Ábrahám and Ginál, 1967). Land use changes from native vegetation to cropping and continuous cropping decreases the organic carbon content of soils (Guo and Gifford, 2002).

The E4/E6 ratio was highest at SGlP1 (Table 2) thereby suggesting the lower quality of organic matter as a result of vegetation and humus transformation driven by soil microbes. The decomposition of humus may be due to the interaction between stable humus and soil microorganisms resulting in the alteration of soil organic matter, which indicates a close correlation between the microorganisms and humus formation (Dou and Wang, 2011). However, at the NSnA, NSnM and AScP sites the E4/E6 values were lower than 5 thereby indicating that the area was characterized by humic acids (Stevenson, 1994).

The soil pH values varied from neutral to alkaline (ranging from 6.97 to 9.57) with the exception of the NChA site which was slightly acidic (Table 2). The significant differences in the EC values and pH values of all the sites can be explained by the different soil types and land use/management practices and the intensity of the agriculture (Assefa *et al*., 2020). However, the EC values at one of the Gleysol pasture sites (SGlP) was lower which may be due to the drainage processes (Tóth *et al*., 2015; Molnár *et al*., 2019).

Explaining the high salt and sodium content of salt-affected soils, the higher Na values in the Solonchak (AScP) site may be due to the shallow ground water table (within one metre) with high amounts of water-soluble salts content, which is close to the surface and this results in salt accumulation on, or close to, the surface of the soil. In Solonetz soils, (NSnA and NSnM) the Na values are lower in comparison to the Solonchak soil (AScP) as the groundwater table is lower (with a level that ranges approximately between 1.5-3.0 m from the soil surface), thereby accumulating a low amount of Na^+ at the soil surface, but forming a "Natric horizon" deeper in the soil (WRB 2014- updated 2015).

The cultivated fields have higher nutrient contents due to the regular fertilization processes, but the main macro elements did not affect significantly the studied microbiological parameters (Fig. 4). However, a PCA analysis indicated the role of P_2O_5 in the differentiation of land use types (Fig. 2). Moreover, the results obtained in this study showed that the effects of soil properties and management practices had an influence on soil microbial activity and community structure. Soil biological processes, such as organic matter decomposition and nutrient cycling are catalysed by enzymes. Thus, changes in enzyme activity may affect soil ecosystem functioning. Enzyme activity is related to soil properties such as, pH, EC (Xie *et al*., 2017) moisture and organic matter content (Jordan *et al*., 1995; Bergstrom *et al*., 1998), P_2O_5, K_2O, Mg, Ca and Na (Gangwar *et al*., 2018; Moreno *et al*., 2022) and it is also influenced by soil management practices (Bolton *et al*., 1985; Bandick *et al*., 1999; Ekenler *et al*., 2003; Acosta-Martínez *et al*., 2008). The values of the microbiological properties (BSR, MBC, DHA and phosphatase) at the Solonchak-pasture (AScP) indicated that the AScP plots were microbiologically more active with regard to the largest microbial community, as indicated by the PLFA results (Tables 3 and 4). Also, the higher SD values suggested a substantial degree of heterogeneity in terms of microbiological activity in the area which may be attributed to the greater root mass on permanent grassy vegetation. The measured soil microbiological parameters are regularly used as indicators for investigating soil health and fertility (Alhameid *et al* 2019; Kennedy and Papendick, 1995; Pankhurst *et al*., 1995; Nielsen and Winding, 2002), and reveal the significant differences between different management practices. The lower microbial activities in both arable lands (NSnA and NChA) may be the result of ploughing which disturbed

and homogenized the soil and decreased the soil microbial activity, while continuous plant coverage resulted in an undisturbed environment, and therefore, an increase in microbial enzyme activity in the pasture and meadow sites (Tejada *et al*., 2006).

The variation in microbial activity among the different land use practices is probably associated with the soil moisture level which played an important role in the diversification of microbial activities (CCA, Fig. 4). Weldmichael *et al*. (2021), reported the positive influence of soil moisture on the BSR of different soil types in Hungary. Also, the role of soil water availability and salinity in the composition of the soil microbial community is relevant in forest systems and coastal soils, respectively (Drenovsky *et al*., 2010; Yan *et al*., 2021). When considering each microbial parameter individually, some significant differences were found among the various land use practices studied. Soil microbial parameters were able to distinguish between an abandoned area and extensive cropping and intensive pasture land (Costa *et al*., 2013), whereas other researchers (Qi *et al*., 2018; Zhu *et al*., 2021) have observed significant changes in both the physical and chemical properties of the soil as well as in the microbial biomass after land use changes. Furthermore Tilston *et al*. (2010) stated that the soil microbial community usually changed to a significant extent in response to the current land use practices.

Our results provided new insights into the relationships of the chemical and microbial properties of salt-affected soils under different land use practices because the PLFAs represent *in situ* microbial community composition and biomass size of soils (Kaur *et al*., 2005). The practice of cultivating Chernozem soils as arable land may decrease the size of its microbial community to a third of the microbial community size of the salt-affected Solonetz and Solonchak meadow and pasture lands.

Fungi play an important role in favourable soil structure (Eash *et al*., 1996). Filamentous fungi are more sensitive to physical disturbances like soil tillage than single celled microbes (Kabir *et al*., 1999) causing a decrease in fungal PLFA and in the fungi to bacteria ratio in our sites. The lower fungi to bacteria ratio values of both arable sites indicate the greater effect of tillage than soil type on the community of soil fungi. The fungal PLFA quantities had their lowest values in Chernozem soil following the other arable land on Solonetz soil which indicated stronger negative effects on Chernozem than on Solonetz soil due to the long-standing tillage on Chernozem soil. Jangid *et al*. (2011) suggested the existence of a lasting impact (more than 50 years) of cultivation history on the soil microbial, mainly bacterial community.

Similarly, the G-negative to G-positive ratio (Table 5) also indicated that degradation processes have taken place in arable lands with higher ratios in arable than pasture and meadow lands. G-negative bacteria have cyclo fatty acids in their cell membranes which can help them to survive in stressed environmental conditions (Guckert *et al*., 1986). Moreover, G-negative bacteria rapidly assimilate the rhizodeposits of grasses (Treonis *et al*., 2004) which may explain the highest G-negative bacterial PLFA concentrations in the AScP and NSnM sites. The low G-negative PLFA concentration in the SGlP sites indicated the strong impact of unfavourable soil chemical properties on the size of the microbial community.

The community size of AM fungi is influenced among others by the presence or absence of a host plant (Karasawa *et al*., 2002) and also, plant available soil P content (Koide, 1991). In our experiment, host plants were grown on each site. The plant available P contents in the soil were higher at arable sites (NSnA and NChA) with low AM fungi PLFA content while in the case of the NSnM and AScP sites, low plant available P content and high AMF PLFA content were measured. These results corroborate the role of AM fungi in plant phosphorus acquisition (Kobae, 2019), however, in the case of the SGlP sites the lowest soil P levels and notably low AMF PLFA contents were measured. The gleysol pasture sites seem to form a transition point between the pasture/meadow and arable sites and the soil of this sampling site was found to be very heterogeneous concerning soil microbial properties, mainly the PLFA content (Table 3). Moche *et al*. (2015) also found low concentrations of G-positive bacteria, Actinobacteria and fungi PLFA markers in Eutric Gleysol in Germany, where they found that soil organic carbon and soil texture had the main influence on the microbial community. This observation was confirmed by our results with the lowest organic matter content being found in Gleysol sampling plots.

Concerning the total PLFA concentrations of the studied plots, the undisturbed pasture and meadow soils had higher values than the arable sites. The regular tillage of arable sites usually decreased the microbial biomass and richness of arable lands (Zornoza *et al*., 2009).

A Bray-Curtis analysis (Fig. 3) resulted in two groups of sampling sites: non-salt-affected (NChA and SGlP) and salt-affected (NSnA, NSnM and AScP). Concerning the effect of vegetation cover of pasture and meadow sites this result is in accordance with Jangid *et al*. (2011) and Rajaniemi and Allison (2009) who found that the plant community was not the main driver in the microbial community pattern. However, land use has a substantial effect on the soil microbial community (Van Leeuwen *et al*., 2017; Drenovsky *et al*., 2010), more so than vegetation and soil properties and the recovery of the damaged microbial community of the crop soil requires several years, mainly in bulk soil (Jangid *et al*., 2011). On the other hand, Bezemer *et al*. (2006) and Lucas-Borja *et al*. (2012) did not find any relationship between the soil microbial community structure and land-use type.

Fuchs *et al*. (2011) investigated the taxonomic distances between the various Hungarian soil types based on the soil forming processes and concluded that salt-affected soils (‘Solonchaks’ and ‘Solonetzs’) formed a well separated

cluster from the other soil classification units, and the soil types are very close to each other with short taxonomic distances, due to the characteristic soil forming processes, such as salt and sodium accumulation. In the case of 'Chernozems' soils, the most dominant soil forming process was humification, with the result that these soils did not form a coherent, distinct taxonomic group, but rather, the different soil types are close to other soil taxonomic units, like 'Gleysols'. These taxonomic distances may be observed based on our investigations as the driving factor is the same, such as land use, but the soils formed two well separated clusters where the most dominant factor was the soil type not the land use, as these soils are far from each other in chemical and physical properties, and the soil forming processes manifesting in soil classification units. However, within one taxonomic soil unit, or as the soils are close to each other, the land use type had a more pronounced effect on soil microbiological properties.

CONCLUSIONS

1. The soil chemical, physical and microbiological properties of five sampling sites were studied with different soil types under different land use practices to understand how the land use practices and soil types affected the soil physical and chemical differences and also to find the main driving factors of soil microbial properties.

2. Principal component analysis of the chemical properties of the soil proved that the sites could be grouped according to the land use and soil type. Cultivating Chernozem soils as arable land could decrease the size of its microbial community to a third of the microbial community size of the salt-affected Solonetz and Solonchak meadow and pasture lands. However, the measured soil chemical parameters were different among sampling sites and P_2O_5 played a key role in site differentiation, the microbial properties were mainly determined by soil moisture content, according to the canonical correspondence analysis results.

3. Based on all of the microbiological properties studied including phospholipid fatty acid, the salt affected soils formed a well separated cluster as opposed to the other soil classification units which were non-salt affected soils. Soil types may be the driving factor as salt-affected soils and non-salt-affected soils are far away from each other in terms of taxonomic distances, for soil groups with short taxonomic distances, land use had more pronounced effects on soil microbiological properties.

4. Continuous plant coverage and the decreased mechanical disturbance of the soil may preserve and/or improve soil function which was proven by our microbial and chemical results. Preserving and enhancing the organic matter content of our soils will improve their microbiological properties.

Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have the appearance of influencing the work reported in this paper.

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