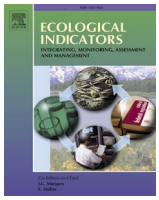
Ecological Indicators 158 (2024) 111545

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind



Original Articles

Response of soil microbiota to various soil management practices in 100-year-old agriculture field and identification of potential bacterial ecological indicator

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ARTICLE INFO

Keywords:
Long-term soil use
Microbial soil community
Microbial indicators

ABSTRACT

The study aimed to comprehensively investigate the structural dynamics of a soil microbial community within a 100-year agricultural field experiment. The experimental design encompasses three distinct soil management practices, namely monoculture, five–year rotation, and random rotation, each with or without the incorporation of legumes and manure. Soil microbial communities were determined by the Next-Generation Sequencing (Illumina MiSeq analysis) of both the V3 and V4 hypervariable regions of the 16S rRNA gene for bacteria and Archaea, as well as ITS1 for fungi. In all soils, dominant bacterial phyla were Proteobacteria, Actinobacteria, Acidobacteria, and Chloroflexi, and dominant orders were Actinomycetales, Rhizobiales, Acidimicrobiales, and Sphingomonadales. However, dominant fungal phyla were Ascomycota, Basidiomycota, and Zygomycota, and dominant fungal orders were Pleosporales, Eurotiales, Hypocreales, or Mortierellales. Compared to fallow land, agricultural soil management affected the microbial community of the soil, reducing the ratio of oligotrophs (e.g. Acidobacteria and Armatimonadetes) to copiotrophs (Actinobacteria or Gemmatimonadetes). Moreover, agricultural soil management contributed to an increased number of plant growth-promoting bacterial groups (PGPB), antagonistic to many fungal (e.g., *Fusarium* spp.) and bacterial pathogens (e.g., Bukholderiales, Xanthomonadales). However, generally in the study, there were no significant differences in microbial communities between monoculture and crop rotations. Moreover, two taxa can be considered as potential indicators of "healthy soil": the nitrifying bacteria *Nitrospira* spp. whose abundance was strongly dependent on nitrogen, potassium, phosphorus, organic carbon, and soil pH, and the PGP fungi of the genus *Mortierellla* which depended mainly on nitrogen and pH. Finally, the genus *Mortierellla* was generally the most abundant in agricultural soils, especially in the five–year rotation with legumes, while fallow soils did not favour these microorganisms. In conclusion, various soil management practices strongly affect the soil microbiota and thus their ability to support land productivity.

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https://doi.org/10.1016/j.ecolind.2024.111545
Received 8 September 2023; Received in revised form 22 December 2023; Accepted 1 January 2024
Available online 8 January 2024

1. Introduction

Agriculture is one of the most important practices affecting the global economy by determining both the food supply and the natural environment (Dobrzyński et al., 2022b; Heyi et al., 2022; Ramdan et al., 2020; Tibbett et al., 2020). Agricultural activities in the long-term have been reported to significantly affect the physical, chemical and biological properties of the soil (Campbell et al., 2013; Gajda et al., 2018; Swedrzynska et al., 2013; Smagacz and Martyniuk, 2023). Soil faunal organisms such as earthworms and nematodes have so far been reported as important biological indicators of soil health, whereas, the importance of soil microorganisms, another very important indicator of soil health, has been poorly studied (Directorate-General for Research and Innovation (European Commission) et al., 2020). The soil microbiota plays one of the crucial roles in the soil ecosystem by determining rates of mineralisation and humification of organic matter (Chiba et al., 2021), biogeochemical cycles of elements (Bielińska and Mocek-Płóciniak, 2012; Dobrzyński et al., 2021; Górska et al., 2014), promotion of plant growth (Dobrzyński et al., 2022b, 2022a; Kulkova et al., 2023; Oleńska et al., 2020; Wróbel et al., 2023), mycorrhization (Chifetete and Dames, 2020), and suppression of phytopathogens (Dobrzyński et al., 2023a; Haas and Défago, 2005).

Specifically, the dominant bacterial taxa of arable soils at the phylum level are Proteobacteria, Actinobacteria, Acidobacteria, Planctomycetes, Chloroflexi, Verrucomicrobia, Bacteroidetes, Gemmatimonadetes, Firmicutes, Armatimonadetes, TM7, and WS2 (Delgado-Baquerizo et al., 2018; Dobrzyński et al., 2021; Fierer et al., 2007). Whereas, the dominant phyla among fungi are the Ascomycota, Basidiomycota, and the Mucoromycota and Zoopagomycota (both formerly members of the „Zygomycota") (Klaubauf et al., 2010; Schoch et al., 2020; Spatafora et al., 2016; Tedersoo et al., 2018).

In recent years, the structure, diversity, and activity of soil microbial communities have been increasingly used as indicators of soil health and production potential. Determining whether changes in soil microbial communities are beneficial or detrimental to plants can help improve the productivity and stability of agroecosystems (Wang et al., 2019). Although there is evidence that various practices of soil management including monoculture, crop rotation, and fallow land determine the soil microbial community (Bielinska & Mocek-Płóciniak, 2012; Dobrzyński et al., 2021), only a few long-term studies have addressed their effects on the composition and taxonomic diversity of the microbial community (Langer and Klimanek, 2006; Gałązka and Grządziel, 2018; Dobrzyński et al., 2023b).

Therefore, the study aims to assess the impact of long-term agricultural soil management on soil microbial communities, which allows to determine whether the microbiota is negatively affected by the management practices used in the studied soil. More specifically, the study determines the physicochemical properties, composition, and taxonomic diversity of prokaryotic and eukaryotic microbial communities in soils grown with potatoes and rye under two different crop rotations - monoculture and fallow land. In addition, the study aims to answer whether potential indicators of 'healthy soil' can be identified by assessing the impact of soil management practices on microbial communities. In turn, the answer based on the determination of correlations between soil management practices, soil chemical properties, and the microorganisms dominating the studied soil will contribute towards sustainable management of the agricultural ecosystem. Notably, with these assumptions, our research fits in 6 with the objectives of important directives including the Green Deal and The EU soil strategy for 2030.

Furthermore, as reported by other authors, microbial communities in relatively shortly-used soils can rapidly revert to their initial state, hence in some cases no change in these communities is observed after several years (Geisseler and Scow, 2014; Wierzchowski et al., 2021). Thus, the research was conducted on a nearly 100-year-old field experiment belonging to the Institute of Agriculture, Warsaw University of Life Sciences. Considering the fact that there are only a few such experiments in the world, e.g. Rothamsted (United Kingdom), Halle (Germany), or Morrow Plots (USA), the study is a unique opportunity to study the relations between soil management practices and microbial community after such a long period of soil use.

2. Materials and methods

2.1. Study area

The soil was collected from a long-term fertiliser experiment conducted at the Departmental Experimental Station of the Warsaw University of Life Sciences in Skierniewice, Poland (51°57′54.8″N, 20°09′27.4″E, 129 m.a.s.l.). In 1923, the uninterrupted and on-going experiments were established on a Luvisol (IUSS Working Group WRB, 2015) with textural classes from loamy sand for the arable horizon, to loam for parent rock. A detailed description of the experiments covering ca. 5 ha of arable land is included in the paper by Mercik and Stępień (Mercik and Stępień, 2005). Briefly, mean annual T° in the last 80 years was 7.9 °C and mean annual precipitation was 528 mm. Overall, in the long-term experiment, there are ten fertiliser combinations with four replicate plots (36 m^2 each). Every year, mineral fertiliser was applied (90 kg N ha^{-1}–ammonium nitrate 26 kg P ha^{-1}–superphosphate; 91 kg K·ha^{-1} –potassium salt), and every 4 years, 2 t CaO ha^{-1}–calcium carbonate was applied. The crop protection agents used are presented in Table 1.

In 2016, after a crop harvest from potato and rye cultivation, the soil was collected from the topsoil (0–30 cm) in three different soil management practices on separate fields (Table 2). During the year, the same mineral fertilisation was used in each experimental variant except for fallow land. Soil samples were taken in 3 replicates with an Egner soil auger, 10 samples for each treatment. Then, representative samples were made for each repetition. The soil for the DNA analysis was immediately frozen at −80 °C. Crop yields were measured as described previously by Mercik and Stepień (2005).

2.2. Laboratory analysis

The physicochemical parameters of the soil were determined for pH with 1 mol KCl and potentiometrically in a H_2O suspension with a 1:2.5 ratio. Total carbon (C) and nitrogen (N) were determined according to the following norms: PN-ISO 10,694 (determination of organic and total C after dry combustion – elementary analysis) and PN-ISO 11,261 (determination of total N – modified Kjeldahl method). Available forms

Table 1
Plant protection products used.

No	Preparation	Action	Substance and dose
1.	Kestrel 200	Protect potatoes against potato beetle	acetamiprid (200 g l^{-1} – 0.17 l ha^{-1})
2.	Karate zeon	Protect potatoes against potato beetle	lambda-cyhalothrin (50 g l^{-1} – 16 l ha^{-1})
3.	Infinito 687.5	Disease protection	propamocarb hydrochloride (625 g l^{-1}); fluopicolide (62.5 g l^{-1} – 1.6 l ha^{-1})
4.	Carial star 500	Disease protection	mandipropamid (250 g l^{-1}); difenoconazole (250 g l^{-1} – 0.6 l ha^{-1})
5.	Banjo forte 400	Disease protection	fluazinam (200 g l^{-1}); dimethomorph (200 g l^{-1} – 0.8 l ha^{-1})
6.	Proman 500/ Metobrom + Racer 250	Protection against weeds (potato fields)	(metobromuron 500 g l^{-1} + flurochloridone 250 g l^{-1}; Proman – 2 l ha^{-1} + Racer – 1 l ha^{-1}).
7.	Bison	Protection against weeds (rye fields)	(diflufenican – 100 g l^{-1} (9.48 %), florasulam 3.75 g l^{-1} and penoxsulam 15 g l^{-1} –1 l ha^{-1}).

Table 2
Experimental variants.

Abbr.	Soil management practice	Crops	Legume
FL	Fallow Land	Not cultivated nor fertilized for several decades	–
RP	Random crop rotation (potato)	Potatoes ver. Hermes, winter wheat ver. Symphony, spring barley ver. Stratus	No
MP	Monoculture of potato	Potatoes ver. Hermes	No
CP	Five–year crop rotation (potato)	Potatoes ver. Hermes, spring barley ver. Stratus, lupinus ver. Sonet, winter wheat ver. Symphony, rye ver. Dańkowskie	Yes
RR	Random crop rotation (potato)	potatoes ver. Hermes, winter wheat ver. Symphony, spring barley ver. Stratus	No
MR	Monoculture of rye	Rye ver. Dańkowskie	No
CR	Five–year crop rotation (rye)	Potatoes ver. Hermes, spring barley ver. Stratus, lupinus ver. Sonet, winter wheat ver. Symphony, rye ver. Dańkowskie	Yes

of potassium (K) and phosphorus (P) were determined with the Egner-Riehm method (Egner and Riehn, 1958).

For each sample, the genomic DNA of the soil was extracted from 1 g of homogenised soil using the Sherlock AX kits (A&A Biotechnology, Gdynia, Poland) including an added enzymatic lysis step and a step of mechanical lysis with zirconia beads by the FastPrep-24 instrument. The procedure of DNA extraction sequencing was precisely described in earlier work by Hewelke et al. (2020). The amplification of the V3-V4 hypervariable region of 16S rRNA gene was carried out by the Q5 Hot Start High-Fidelity 2X Master Mix (New England Biolabs, Ipswich, MA, USA). The following specific primers were used for reaction: 341F and 785R (Klindworth et al., 2013) extended by an adaptor sequence, thus enabling indexing using Nextera XT Index primers in the second PCR reaction (Illumina Inc., San Diego, CA, USA).

The amplification of the ITS1 hypervariable region for fungi metabarcoding was done by the Q5 Hot Start High-Fidelity 2X Master Mix (New England Biolabs, Ipswich, MA, USA). The following specific primers were used for PCR: ITS1F12 and 5.8S (Schmidt et al., 2013; "Vilgalys Mycology Lab – Duke University | Duke Mycology," n.d.) with an extension of an adaptor sequence, allowing for indexing to be carried out in the second PCR sequence. For this purpose, a V2 sequencing kit on a MiSeq sequencer was used (Illumina Inc., San Diego, CA, USA) along with 2x250 paired end reads (in Genomed S.A., Warsaw). Secondary analysis, which involved automatic demultiplexing and FASTQ generation, was conducted with the MiSeq Reporter Software v2.6. Cutadapt version 1.9 (Martin, 2011) that was used to trim the adapter and primer sequences, joining them with a fastq-join algorithm (Aronesty, 2011). During the laboratory preparation, a negative control was used, however, due to the shortages in the obtained library, it was not sequenced. The removal of the adapter and primer sequences was performed with the Cutadapt program.

The QIIME software package (Caporaso et al., 2010) allowed for further analyses. They comprised the following stages: the pairs of readings were joined by the fastq-join program with a minimal "tab" with a length of 10 bases, chimeric sequences were identified and removed using USEARCH, and open-reference clustering (OTU picking) was carried out at the level of 97 % sequence similarity with the uclust algorithm, providing a taxonomy based on the UNITE database. Most likely, the high percentage of unassigned sequences results from the open-reference clustering and the lack of a referencial sequence in the UNITE database.

Open-reference operational taxonomic unit (OTU) picking was performed based on the GreenGenes v13_8 database (DeSantis et al., 2006) for the 16S analysis as well as the UNITE version of the database from 2-03-2015 (Kõljalg et al., 2013) for the ITS1 analysis. Chimaeras were detected with the ChimeraSlayer (Haas et al., 2011) and USEARCH (Edgar, 2010) for the 16S and ITS1 analyses respectively, with the removal of singletons and OTUs characterised by fewer than 10 sequences.

2.3. Statistical and bioinformatics analyses

Basic tests on the physicochemical properties and microbiological diversity were done by one-way analysis of variance - homogeneous groups were distinguished by Tukey's test for $\alpha \leq 0.05$, using the Statgraphics ver.plus 4.1 program. Principal component analysis (PCA) was used to investigate the relationships between the studied variables (i.e., microorganism taxa) and multivariate differences between the studied objects (i.e., crop rotations). Genus-level dominants were used for the determination of potential microbiological indicators of soil health. The PCA was performed using the Statistica 13 software. The results are presented as biplots where the first (PC1) and the second (PC2) principal components are exhibited, as well as the percentage of explained variability for each PC. Alpha diversity was analysed using the observed OTUs and the Chao-1 richness estimator (Chao, 1984).

3. Results

3.1. Soil chemical properties and yields

The least favourable soil chemical properties were recorded in the fallow land (FL – Table 3). The most favourable chemical properties characterised the soils collected from the five–year rotation (CP and CR), organically fertilised and cultivated with legumes. These soils contained more organic C, N, and P than soils from the two other soil management practices (random crop rotation and monoculture). Soils from the monocultures and random crop rotation contained similar amounts of C and N, however, the monocultures contained higher amounts of available forms of K and P and showed a higher pH than the soils from the random crop rotation. Besides, regarding the effect of the plant on the chemical composition, the soil from the rye crop generally contained slightly more C and N than the soil from the potato crop, but it was not statistically significant. In terms of yields, both potato and rye had the highest values in five–year rotations. However, in the case of rye, the obtained values were statistically significant only in comparison to the yields from monoculture (Table 3).

3.2. Soil microbiota

For prokaryotes and eukaryotes, the reads obtained from the sequencing process of the studied soils are presented in supplement. Overall, the metagenomic analyses of the soil showed that the agricultural soil management increased the α-diversity (Chao index) of prokaryotic and eukaryotic organisms compared to the fallow soil (FL) which had not been cultivated for almost 100 years. Similar results were found for the OTUs number (Table 4). For the prokaryotic group, the highest α-diversity was observed in RP and CR. For the eukaryotic group, the highest diversity was observed in CP as well as in MR. Overall, the results indicate that soil management practice had a greater impact on index values than plant species (Table 4).

3.2.1. Structure of bacterial community

At the phylum level, all analysed soils, regardless of the agricultural management practice, were dominated by Proteobacteria (26.96–28,66 %), Actinobacteria (11.85–25.95 %), Acidobacteria (13.62–16.76 %), and Chloroflexi (10.94–14.42 %). In terms of the relative abundance of Proteobacteria, Chloroflexi, and Verrucomicrobia, there was no significant difference between the soils from management practices compared to fallow land (Fig. 1a, Table 5). Although, compared to fallow land, in all agriculturally managed soils, a significant increase was noted in the

Table 3
Chemical properties of soils and yields (letters indicate statistically different groups - Tukey test at $\alpha \leq 0.05$).

Abbr.	Soil Mangement practice	pH		total $C_{org.}$	N	C:N	available K	P	yields
		KCL	H_2O	g kg^{-1}			mg kg^{-1}		t·ha^{-1}
FL	fallow land	5.1[a]	5.5 [a]	4.21[a]	0.41[a]	10.3[b]	31.6[a]	18.4[a]	-
soil from potato cultivation									
RP	random	5.2[a]	5.7[a]	4.82[a]	0.49[a]	9.8[b]	60.7[b]	41.9[b]	20,03[b]
MP	monoculture	6.3[b]	6.5[b]	4.78[a]	0.48[a]	10.0[b]	74.1[c]	56.7[c]	18,26[a]
CP	crop rotation	6.6[c]	6.8[c]	7.18[b]	0.69[b]	10.4[b]	84.2[d]	49.8[b]	31,18[c]
soil from rye cultivation									
RR	random	5.1[a]	5.6[a]	5.22[a]	0.59[a]	8.8[a]	55.3[b]	34.9[b]	4,01[b]
MR	monoculture	6.4[b]	6.6[b]	5.32[a]	0.51[a]	10.4[b]	60.3[c]	59.8[c]	3,44[a]
CR	crop rotation	6.8[c]	7.0[c]	6.95[b]	0.66[b]	10.5[b]	81.9[d]	48.1[b]	4,56[b]

Table 4
Observed mean richness (OTUs number) and diversity (estimated by Chao1 index) of prokaryotes and eukaryotes estimated by metagenomic analysis (97 % OTU similarity) (letters indicate statistically different groups - Tukey test at $\alpha \leq 0.05$), n = 3.

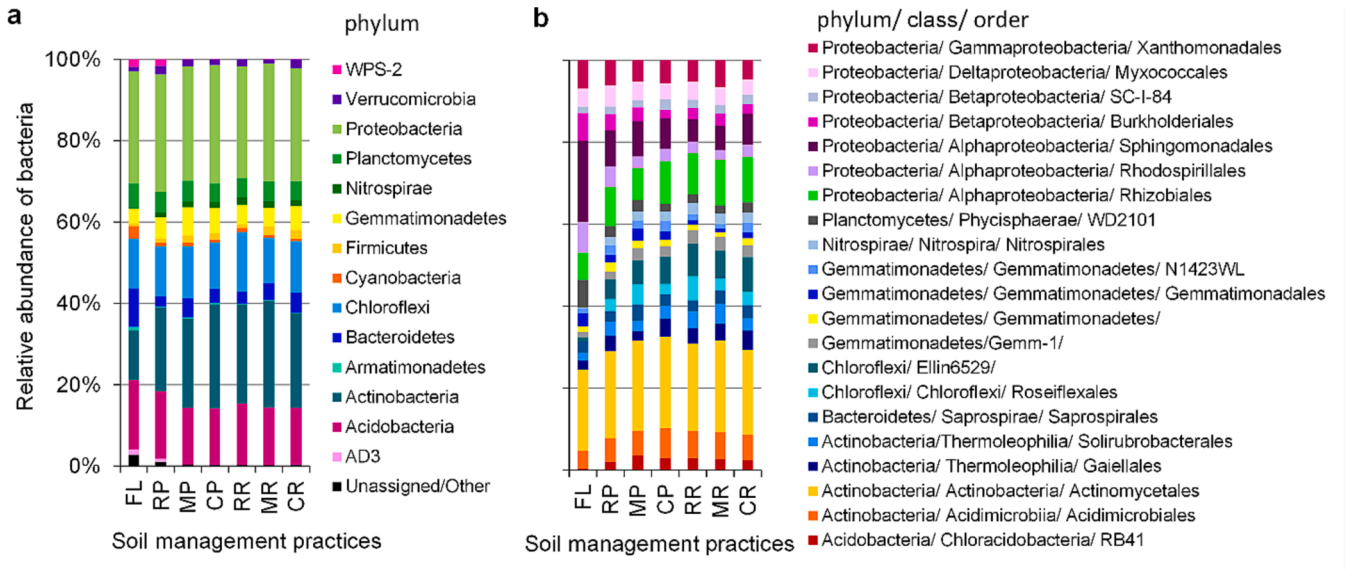
Abbr.	Soil management practise	Prokaryotes OTUs average	Chao1 average	Eukaryotes OTUs average	Chao1 average
FL	fallow land	2162.7[a]	1794.9[a]	1327.2[a]	1551.7[a]
soil from potato cultivation					
RP	random	3444.9[c]	4463.4[c]	2174.1[b]	2348.9[b]
MP	monoculture	3376.4[b]	4276.2[b]	2229.6[b]	2568.0[b]
CP	crop rotation	3412.2[b]	4411.4[b]	2339.2[c]	2595.4[b]
soil from rye cultivation					
RR	random	3174.1[b]	4072.7[b]	2172.5[b]	2500.7[b]
MR	monoculture	3245.8[b]	4073.3[b]	2360.8[c]	2634.2[c]
CR	crop rotation	3522.8[c]	4556.1[c]	2205.8[b]	2595.5[b]

relative abundance of heterotrophs including Actinobacteria, Gemmatimonadetes, Firmicutes, and autotrophic Nitrospira. However, in comparison with the other treatments, the relative abundance of OTUs belonging to WPS-2 (or Eremiobacterota), Planctomycetes, Cyanobacteria, Bacteroidetes, Armatimonadetes, and AD3 was significantly higher in the soil from FL. Moreover, compared to the other treatments, the significantly highest relative abundance of the phylum Acidobacteria was noted in the soil from FL and RP (Fig. 1a, Table 5).

In terms of the dominants of bacterial order, Actinomycetales (9.59–17.43 %), Rhizobiales (3.20– 8.54 %), Acidimicrobiales (2.07–5.77 %), and Sphingomonadales (4.28–9.56 %) were the most abundant (Fig. 1b, Table 6). Compared to the FL, significantly more sequences assigned to Acidimicrobiales, Actinomycetales, Gaiellales, Solirubrobacterales, Roseiflexales, Nitrospirales, Rhizobiales, o_SC-I-84 (belonging to Betaproteobacteria), Myxococcales, and Xanthomonadales were recorded in the tilled soils, while significantly more sequences assigned to Rhodospirillales, Burkholderiales, and o_WD2101 (Phycisphaerae class) were noted in the FL soil compared to other treatments.

Overall, in the several cases described above, differences were noted in relative abundance between agricultural soil management and fallow soils. However, no trend was observed to indicate that the relative abundance of a particular phylum and order of bacteria is related to a particular soil management practice or a particular plant.

Fig. 2a and 2b present the results of the PCA analysis studying mutual dependencies between the abundance of particular prokaryotic taxa at the phylum level and the agricultural management practices. Overall, the PCA analysis confirms that the bacterial communities in agricultural



Soil management practices: FL - fallow land, RP - random crop rotation with potato, MP - monoculture of potato, CP - crop rotation after potato, RR - random crop rotation with rye, MR - monoculture of rye, CR - crop rotation system after rye.

OTUs are defined at a 97% sequence identity threshold. Major taxa (phylum level) and candidate taxonomic groups with a relative sequence abundance of ≥ 1.0% were detected in at least one of the soil management practices.

Fig. 1. Relative abundance of dominant bacterial phyla (a) and classes/orders of the dominant phyla (b) in soils collected from different soil management practices.

Table 5
Dominant phyla of procaryota (relative abundance ≥ 1 % in at least one of the samples) in the experimental treatments (n = 3 per each soil sample, α ≤ 0,05, *t*-test).

Taxa	FL	RP	MP	CP	RR	MR	CR
Unassigned; Other	2.7^{b}	1.01^{a}	0.38^{a}	0.26^{a}	0.28^{a}	0.21^{a}	0.29^{a}
p_AD3	1.35^{b}	0.73^{a}	0.00^{a}	0.04^{a}	0.00^{a}	0.00^{a}	0.14^{a}
Acidobacteria	16.76^{b}	16.30^{b}	13.69^{a}	13.62^{a}	14.87^{a}	14.01^{a}	13.7^{a}
Actinobacteria	11.85^{a}	20.39^{b}	21.67^{b}	25.22^{b}	24.12^{b}	25.95^{b}	22.83^{b}
Armatimonadetes	0.94^{b}	0.25^{a}	0.27^{a}	0.27^{a}	0.17^{a}	0.18^{a}	0.21^{a}
Bacteroidetes	9.27^{b}	2.47^{a}	4.74^{a}	3.46^{a}	2.91^{a}	4.16^{a}	4.88^{a}
Chloroflexi	11.96^{a}	11.97^{a}	12.49^{a}	11.1^{a}	14.42^{a}	10.94^{a}	12.43^{a}
Cyanobacteria	3.03^{b}	0.96^{a}	0.92^{a}	0.85^{a}	1.06^{a}	0.76^{a}	0.69^{a}
Firmicutes	0.53^{a}	1.01^{b}	1.68^{b}	1.42^{b}	0.82^{b}	2.05^{b}	2.07^{b}
Gemmatimonadetes	3.73^{a}	5.17^{b}	6.88^{b}	6.07^{b}	4.9^{b}	4.52^{b}	5.76^{b}
Nitrospirae	0.1^{a}	1.11^{b}	1.39^{b}	1.44^{b}	1.72^{b}	1.65^{b}	1.48^{b}
Planctomycetes	5.95^{b}	4.95^{a}	5.01^{a}	4.49^{a}	4.71^{a}	4.72^{a}	4.61^{a}
Proteobacteria	26.96^{a}	28.45^{a}	27.73^{a}	28.66^{a}	27.14^{a}	28.66^{a}	27.33^{a}
Verrucomicrobia	1.15^{a}	2.04^{a}	1.7^{a}	1.36^{a}	1.71^{a}	1.04^{a}	2.17^{a}
WPS-2	1.83^{b}	1.62^{a}	0.01^{a}	0.03^{a}	0.00^{a}	0.01^{a}	0.01^{a}

Table 6
Dominant phyla/class/orders of bacteria (relative abundance ≥ 1 % in at least one of the samples) in the experimental treatments (n = 3 per each soil sample, α ≤ 0,05, *t*-test).

Bacterial taxa	FL	RP	MP	CP	RR	MR	CR
k_Bacteria;p_Acidobacteria;c_[Chloracidobacteria];o_RB41	0.18^{a}	1.32^{b}	2.77^{b}	2.23^{b}	2.28^{b}	2.08^{b}	1.76^{b}
k_Bacteria;p_Actinobacteria;c_Acidimicrobiia;o_Acidimicrobiales	2.07^{a}	4.00^{b}	4.59^{b}	5.77^{b}	5.10^{b}	5.06^{b}	4.72^{b}
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Actinomycetales	9.59^{a}	14.40^{b}	17.06^{b}	17.43^{b}	16.37^{b}	17.24^{b}	15.40^{b}
k_Bacteria;p_Actinobacteria;c_Thermoleophilia;o_Gaiellales	1.06^{a}	2.66^{b}	1.77^{a}	3.42^{b}	2.95^{b}	3.07^{b}	3.54^{b}
k_Bacteria;p_Actinobacteria;c_Thermoleophilia;o_Solirubrobacterales	0.93^{a}	2.28^{b}	1.94^{b}	2.43^{b}	3.08^{b}	3.73^{b}	2.25^{b}
k_Bacteria;p_Bacteroidetes;c_[Saprospirae];o_[Saprospirales]	1.30^{a}	1.71^{a}	3.19^{c}	2.26^{b}	2.10^{b}	2.52^{b}	2.39^{b}
k_Bacteria;p_Chloroflexi;c_Chloroflexi;o_[Roseiflexales]	0.00^{a}	2.06^{b}	3.80^{b}	1.94^{b}	4.54^{c}	2.30^{b}	2.43^{b}
k_Bacteria;p_Chloroflexi;c_Ellin6529;o_	0.51^{a}	3.25^{b}	4.57^{b}	5.13^{b}	6.13^{c}	5.19^{b}	6.35^{c}
k_Bacteria;p_Gemmatimonadetes;c_Gemm-1;o_	0.60^{a}	1.30^{a}	2.27^{b}	1.99^{b}	2.51^{b}	2.57^{b}	2.12^{b}
k_Bacteria;p_Gemmatimonadetes;c_Gemmatimonadetes;o_	0.72^{a}	1.50^{b}	1.33^{b}	1.24^{b}	1.11^{a}	0.90^{a}	1.33^{b}
k_Bacteria;p_Gemmatimonadetes;c_Gemmatimonadetes;o_Gemmatimonadales	1.53^{b}	1.24^{b}	2.31^{b}	1.59^{b}	0.81^{a}	0.64^{a}	1.13^{b}
k_Bacteria;p_Gemmatimonadetes;c_Gemmatimonadetes;o_N1423WL	0.57^{a}	1.68^{b}	1.57^{b}	2.00^{b}	1.14^{b}	0.86^{a}	1.77^{b}
k_Bacteria;p_Nitrospirae;c_Nitrospira;o_Nitrospirales	0.11^{a}	1.31^{b}	1.68^{b}	1.73^{b}	2.10^{b}	1.96^{b}	1.76^{b}
k_Bacteria;p_Planctomycetes;c_Phycisphaerae;o_WD2101	3.28^{b}	1.82^{a}	2.15^{a}	1.52^{a}	1.56^{a}	1.57^{a}	1.87^{a}
k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Rhizobiales	3.20^{a}	6.53^{b}	6.05^{b}	8.12^{b}	7.85^{b}	8.54^{b}	8.33^{b}
k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Rhodospirillales	3.62^{b}	3.37^{a}	2.23^{a}	2.36^{a}	2.10^{a}	1.86^{a}	2.24^{a}
k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Sphingomonadales	9.56^{b}	5.96^{a}	6.62^{a}	5.83^{a}	4.28^{a}	4.58^{a}	5.57^{a}
k_Bacteria;p_Proteobacteria;c_Betaproteobacteria;o_Burkholderiales	3.27^{b}	2.76^{a}	2.67^{a}	1.69^{a}	2.11^{a}	2.30^{a}	1.75^{a}
k_Bacteria;p_Proteobacteria;c_Betaproteobacteria;o_SC-I-84	0.69^{a}	1.24^{b}	1.38^{b}	1.87^{b}	1.49^{b}	1.62^{b}	1.82^{b}
k_Bacteria;p_Proteobacteria;c_Deltaproteobacteria;o_Myxococcales	2.19a	3.47^{b}	3.44^{b}	3.15^{b}	3.39^{b}	3.26^{b}	2.72^{b}
k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Xanthomonadales	3.35a	4.23^{b}	4.09^{b}	4.36^{b}	4.04^{b}	5.08^{b}	3.52^{b}

soils are significantly different from those from FL. Similarly to the results shown in Table 5, the PCA analysis demonstrates that the bacterial communities in the agricultural soils were similar to each other (Fig. 2a and 2b). The first primary component (PC1) was decisive in explaining total variance (64.57 %), and the taxa P1 (Unassigned; Other), P3 (Acidobacteria), P5 (Armatimonadetes), P6 (Bacteroidetes), P8 (Cyanobacteria), P12 (Planctomycetes), and two candidate taxa for phyla (P15 [WPS-2 (Candidatus Eremiobacteraeota)] and P2 [AD3 (Candidatus Dormibacteraeota)]) were strongly correlated with PC1; they were also mutually correlated with one another in a close. Moreover, the soil in RR was linked and abundantly colonised by the taxa of the P7 (Chloroflexi) and P14 (Verrucomicrobia) phyla (Fig. 2a).

At the order level, the FL was the most different treatment of all (Fig. 2b). Besides, a separate cluster was also formed by bacterial populations from the RR and MR. Notably, the correlations of the MP and 010 (an unsigned order included in Gemmatimondaletes) confirm the largest, but non-statistically significant (according to the Tukey test) abundance of this order in the MP treatment (Table 6).

In general, as in the case of relative abundance, no considerable differences were observed between different soil management practices. However, in the case of plants, there was only a cluster of the MR and RR samples that originated from the same plant (rye).

3.2.2. *Structure of fungal community*

The majority of OTUs were classified as unknown fungal taxa at the phylum level, regardless of the experimental treatment (Fig. 3a, Table 7), with a relative abundance from 48.8 % to 65.1 %. Classified sequences were assigned to 3 fungal phyla and 3 phyla candidates. Overall, regardless of the experimental treatment, Ascomycota were found to be dominant with the highest abundance in the FL (nearly 40 %). The relative abundance of Ascomycota decreased in agricultural soils and was the lowest in the case of MR and CP (Fig. 3a, Table 7). Such results suggest that the phylum Ascomycota may be linked to non-agricultural soils. The highest number of sequences assigned to the phylum Basidiomycota was observed in the CP (4.7 %) as well as in the FL (4.3 %) (Fig. 3a, Table 7). However, compared to the FL (3.82 %), higher relative number of OTUs from the phylum Zygomycota were recorded in agricultural soils (Fig. 3a, Table 7), indicating that their abundance is associated with agrotechnical practices. Additionally, the MP and RR treatments were characterised by a considerable abundance of the taxon F2 (Other Fungi).

In terms of dominants at the order level, compared to the other treatments, significantly the largest abundances of the orders Pleosporales, Helotiales (the phylum Ascomycota), and Tremellales (the phylum Basidiomycota) were found in the soil samples from the FL. In contrast, compared to the FL, higher relative abundance of Mortierellales was observed in agricultural soil management. The patterns

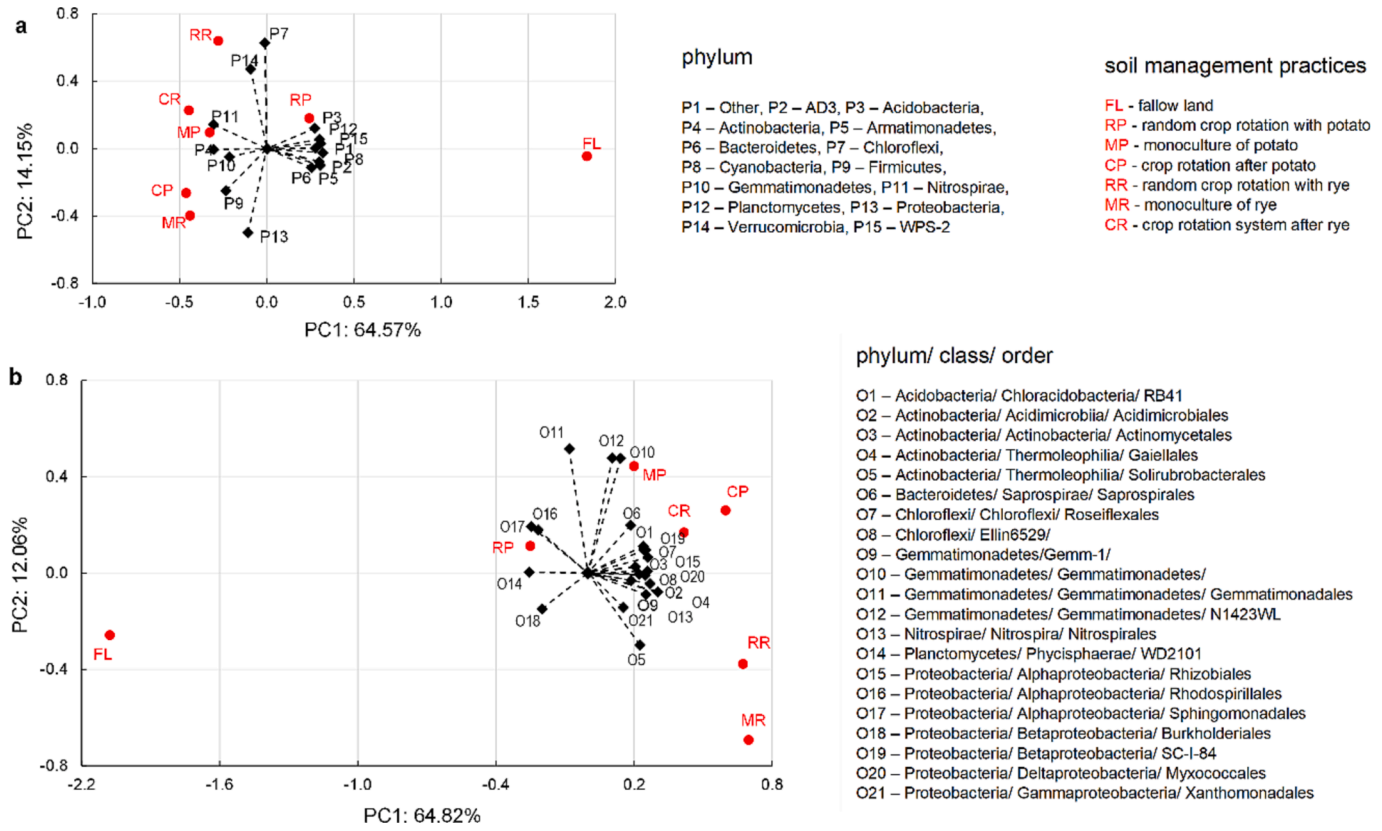
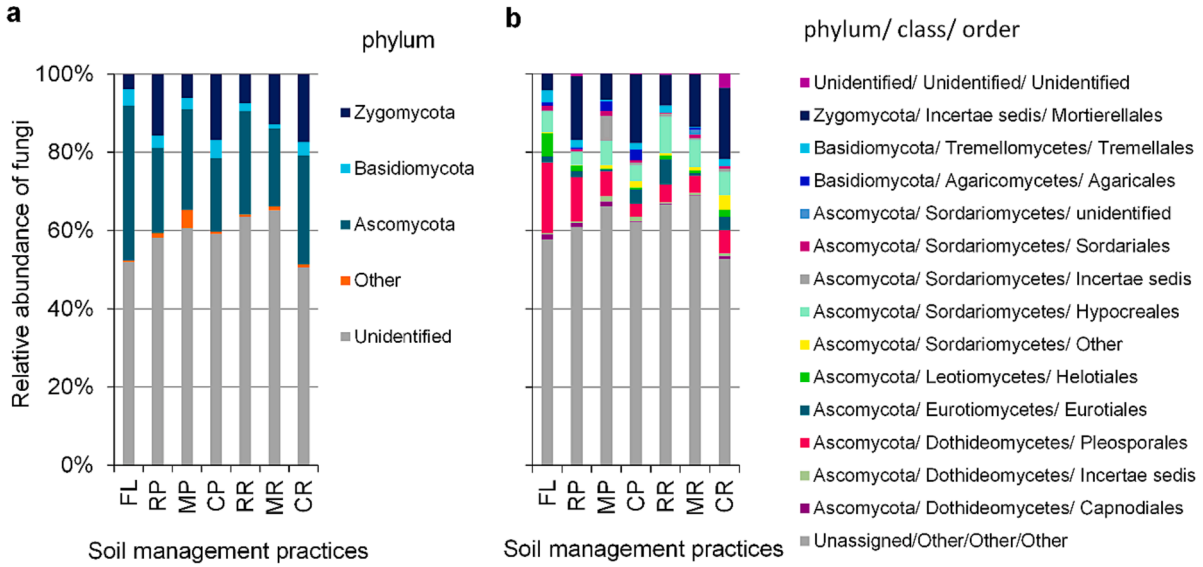


Fig. 2. Principal component analysis (PCA) of the abundance (black rhombuses) of prokaryotic taxa at the phylum level (a) and at the order level (b) (minimum > 1 % abundance in at least one treatment), and of the soil management practices.



Soil management practices: FL - fallow land, RP - random crop rotation with potato, MP - monoculture of potato, CP - crop rotation after potato, RR - random crop rotation with rye, MR - monoculture of rye, CR - crop rotation system after rye.

OTUs are defined at a 97% sequence identity threshold. Major taxa (phylum level) and candidate taxonomic groups with a relative sequence abundance of ≥ 1.0% were detected in at least one of the soil management practices.

Fig. 3. Relative abundance of dominant fungal phyla (a) and classes/orders of the dominant phyla (b) in soils collected from different soil management practices.

above are consistent with those obtained for the relative abundance at the phylum level. Besides, the soil from the MP was also strongly colonised by taxa from the order Incertae sedis (the class Dothideomycetes). While, compared to all treatments, the largest population of the order Eurotiales was recorded in the RR (Table 8). It is also worth mentioning that, compared to the other treatments, the order

Table 7
Relative abundance of dominant fungal phyla in soils collected from different soil management practices (n = 3 per each soil sample, α ≤ 0,05, *t*-test).

Phyla	FL	RP	MP	CP	RR	MR	CR
Unidentified	51,94[a]	57,88[a]	60,64[a]	59,05[a]	63,26[a]	65,08[a]	48,78[a]
Other Fungi	0,37[a]	1,17[a]	4,52[b]	0,68[a]	0,63[a]	1,07[a]	0,85[a]
Ascomycota	39,6[c]	21,58[b]	25,87[b]	18,69[a]	26,3[b]	19,81[a]	26,84[b]
Basidiomycota	4,26[c]	3,26[b]	2,83[a]	4,71[c]	2,06[b]	1,06[a]	3,35[b]
Zygomycota	3,82[a]	15,57[c]	6,11[b]	16,78[c]	7,47[b]	12,85[b]	16,8[c]

Table 8
Dominant phyla/class/orders of fungi (relative abundance ≥ 0,01 % in at least one of the samples) in the experimental practices (n = 3 per each soil sample, α ≤ 0,05, *t*-test).

Fungal taxa	FL	RP	MP	CP	RR	MR	CR
Unassigned; Other; Other; Other	57,45[a]	60,15[a]	63,03[a]	61,73[a]	52,21[a]	67,99[a]	52,20[a]
Other; Other; Other	0,41[a]	1,22[a]	4,70[b]	0,71[a]	0,91[a]	1,12[a]	0,89[a]
p_Ascomycota;c_Dothideomycetes;o_Capnodiales	1,26[a]	0,98[a]	1,17[a]	0,18[a]	0,81[a]	0,18[a]	0,80[a]
p_Ascomycota;c_Dothideomycetes;o_Incertae sedis	0,36[a]	0,35[a]	1,33[a]	1,13[a]	0,66[a]	0,63[a]	0,66[a]
p_Ascomycota;c_Dothideomycetes;o_Pleosporales	18,00[b]	11,22[a]	6,01[a]	3,25[a]	5,85[a]	4,18[a]	5,83[a]
p_Ascomycota;c_Dothideomycetes;o_unidentified	0,00[a]	0,00[a]	0,00[a]	0,01[a]	0,00[a]	0,01[a]	0,00[a]
p_Ascomycota;c_Eurotiomycetes;o_Eurotiales	1,46[a]	1,56[a]	0,53[a]	3,68[b]	3,32[b]	0,72[a]	2,32[a]
p_Ascomycota;c_Leotiomycetes;o_Helotiales	5,97[b]	1,38$_a$	0,21[a]	0,52[a]	1,73[a]	0,62[a]	1,69[a]
p_Ascomycota;c_Sordariomycetes; Other	0,18[a]	0,09[a]	0,80[a]	1,69[b]	3,77[b]	0,94[a]	3,72[b]
p_Ascomycota;c_Sordariomycetes;o_Hypocreales	5,44[b]	3,33[a]	5,95[b]	3,96[a]	5,91[b]	6,92[b]	5,91[b]
p_Ascomycota;c_Sordariomycetes;o_Incertae sedis	0,11[a]	0,21[a]	6,04[b]	0,56[a]	0,82[a]	0,41[a]	0,82[a]
p_Ascomycota;c_Sordariomycetes;o_Sordariales	1,19[a]	0,52[a]	1,18[a]	0,64[a]	0,63[a]	0,87[a]	0,61[a]
p_Ascomycota;c_Sordariomycetes; o_unidentified	0,00[a]	0,00[a]	0,00[a]	0,04[a]	0,01[a]	1,28[a]	0,01[a]
p_Basidiomycota;c_Agaricomycetes;o_Agaricales	0,97[a]	0,39[a]	2,43[b]	2,84[b]	0,08[a]	0,54[a]	0,08[a]
p_Basidiomycota;c_Tremellomycetes;o_Tremellales	3,01[c]	1,91[b]	0,28[a]	1,51[b]	1,74[b]	0,22[a]	1,74[b]
p_Zygomycota;c_Incertae sedis;o_Mortierellales	4,18[a]	16,13[b]	6,33[b]	17,50[b]	17,96[b]	13,31[b]	17,96[b]
p_unidentified;c_unidentified;o_unidentified	0,01[a]	0,55[a]	0,02[a]	0,06[a]	3,58[b]	0,07[a]	3,57[b]

Hypocreales was significantly lowest in the CP and RP (see Table 8).

Fig. 4a demonstrates that fungal taxa at the phylum level were weakly mutually correlated. However, strong positive correlations were observed between the Ascomycota (F3) and the FL, confirming the results of the relative abundance. On the other hand, strong negative correlations were observed between the taxa F1 (Unassigned Fungi) and F4 (Basidiomycota). Besides, the PCA analysis confirmed that the soils from the RP and CP were similar in terms of the taxonomic diversity of fungi.

Fig. 4b shows the PCA analysis at the order level, which indicated that the soil from the MP was characterised by the highest distinctiveness in terms of the abundance of different eukaryotic taxa. Finally, the soils from the FL and RP were the most similar in terms of taxa abundance at order level. They were primarily colonised by the fungi from the taxa T5 (Pleosporales) and T8 (Helotiales). Moreover, the PCA analysis showed positive correlations between the CR and taxa T8 (Helotiales) and T15 (Tremellales).

In general, similarly to the bacterial community, in most cases robust differences were found between the fungal community colonising the FL and various soil management types. Nevertheless, in terms of the order level, the FL and RP fungal communities were strongly correlated.

3.2.3. Relationship between the relative abundance of potential microbiological health indicators and the properties of the soil from management practices

For bacteria, the FL was the most numerously inhabited by *Sphingomonas* spp. and the least by *Nitrospira* spp. and *Rhodoplanes* spp. (Table 9). The PCA analysis indicated a positive relation between the abundance of the genus *Nitrospira* in the soil and the C, N, and both available K and P contents, as well as pH value (Fig. 5a).

In terms of fungi, there was a strong positive relationship between the abundance of the genera *Fusarium* and *Articulospora*, which were also negatively correlated to P contents (Fig. 5b). These fungi were most abundant in the FL soils where P and K were the lowest (Table 10).

4. Discussion

The ecology of microorganisms in agriculturally managed soils is still relatively poorly understood. Therefore, the research aims to evaluate the effects of fallow land (as a control), monocultures, and various crop rotations on bacterial and fungal communities of the soil. Importantly, there are currently only a few similar experiments in Europe, for example in Rothamsted (United Kingdom) carried out since 1843 and in Halle (Germany) carried out since 1894. Hence, a study conducted on such a long–term field experiment with monocultures and different types of crop rotations allowed for a unique assessment of soil microbial communities.

4.1. Soil chemical properties and yields

The results are in agreement with an earlier study by Stępień and Kobiałka (2019) and Dobrzyński et al. (2021) who also conducted studies in the same 100–year-old experimental station. These authors showed higher values of TC and TN in crop rotation in comparison to monoculture soils. Importantly, also Congreves et al. (2017) obtained similar patterns; the authors observed higher soil organic carbon (SOC) and TN content in maize–soybean–wheat rotation soil in comparison with soli from a monoculture of maize at an 11–year–old long–term fertilisation experiment located in Canada. Probably, this phenomenon is a result of a higher amount of crop residues in five–year crop rotations.

Moreover, the studied soil was also acidic, weakly humic, and, above all, very poor in available forms of K and P. Similar results for this treatment were obtained in previous studies of Mercik et al. (2000) and Szara et al. (2017).

In terms of yield results, whose values were higher in rotations than monoculture, it is in line with the previous studies (Chahal et al., 2021; Marini et al., 2020; Mercik and Stępień, 2005).

4.2. Soil microbial communities

In our study, a robust higher Chao1 (α-diversity) index was recorded

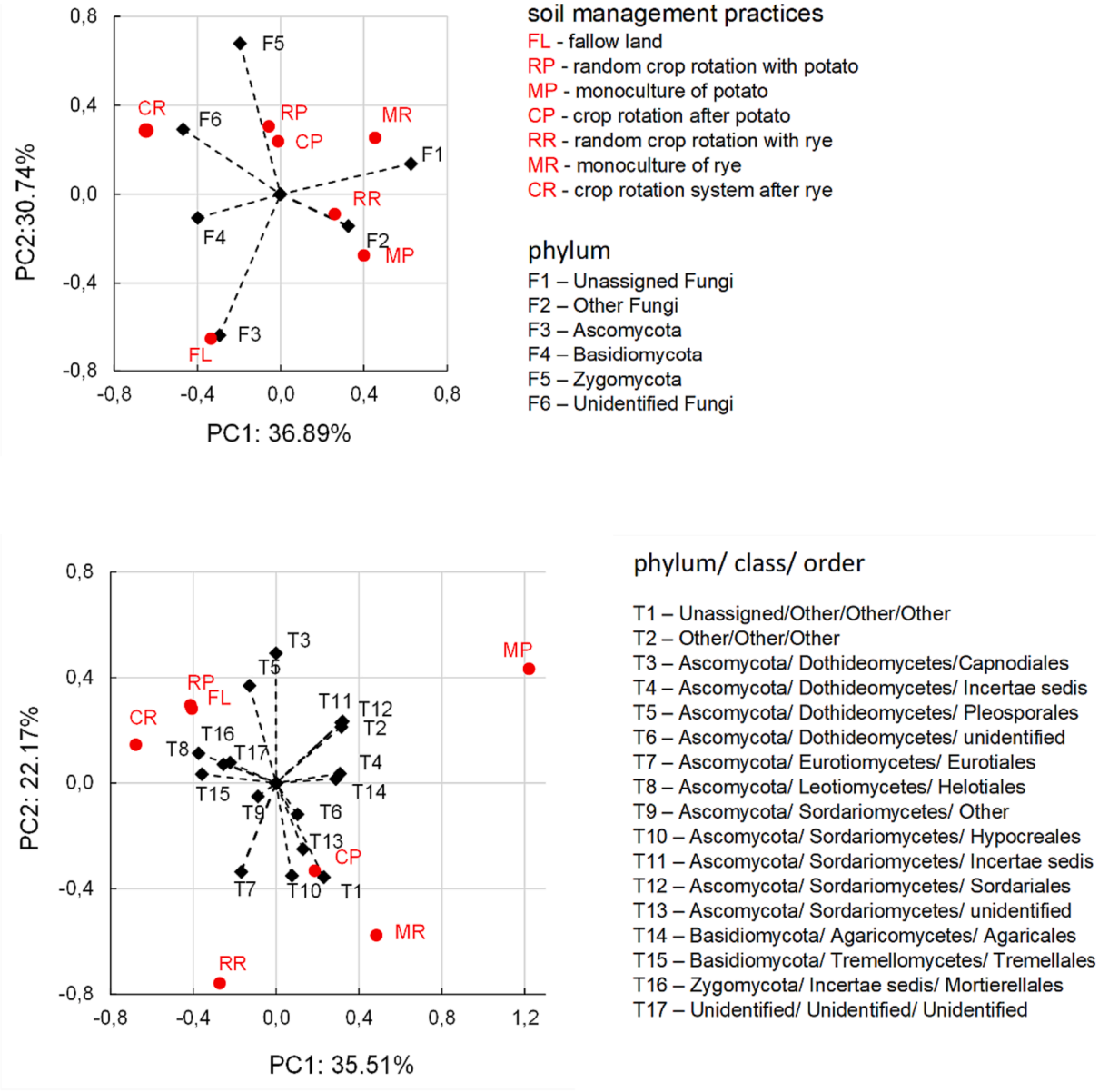


Fig. 4. Principal component analysis (PCA) of fungal taxa abundance (black rhombuses) and soil management practices (red circles).

Table 9
Dominant genera of bacteria (relative abundance ≥ 1 % in at least one of the samples) in the experimental treatments (n = 3 per each soil sample).

Bacteria/Genera	FL	RP	MP	CP	RR	MR	CR
Sphingomonas spp.	3,03[b]	0,36[a]	0,37[a]	0,25[a]	0,37[a]	0,23[a]	0,39[a]
Rhodoplanes spp.	0,64[a]	1,31[a]	1,07[a]	1,42[a]	1,56[a]	1,13[a]	1,68[a]
Nitrospira spp.	0,02[a]	0,56[a]	0,72[a]	0,66[a]	0,63[a]	1,01[a]	0,90[a]

in the agriculturally managed soils compared to the fallow land (for both prokaryotes and fungi), which is consistent with a study conducted by Alami et al. (2021). These authors noted a higher Chao1 index and more OTUs in monoculture of *Fritillaria thunbergii* (2 year-old, monoculture of cabbage (2 years), and monoculture of *Polygonum multiflorum* (2 and 6 year-old monoculture). These patterns can result from the fact that, compared to FL, higher contents of K and P (some of the most important nutrients for microbial growth) were observed in all soil management practices.

Our study also reports no significant differences in the Chao1 index and the OTUs number between the soils from crop rotations and monocultures. To date, in literature, opinions on the influence of soil management practices on OTU numbers and soil microbial diversity indices are divergent. For example, Yin et al. (2010) reported lower values of the Shannon-Weaver index and richness in soil from a wheat-soybean rotation compared to a wheat monoculture. Similar patterns were noted by Mayer et al. (2019). However, a meta-analysis of bacterial diversity in variably managed soils found that diversity values in soils from crop rotation were higher than in soils from monocultures (Venter et al., 2016). The lack of differences in the Chao1 and OTUs indexes in the agriculturally managed soils may result from a relatively slight difference in nutrient content of the studied soils.

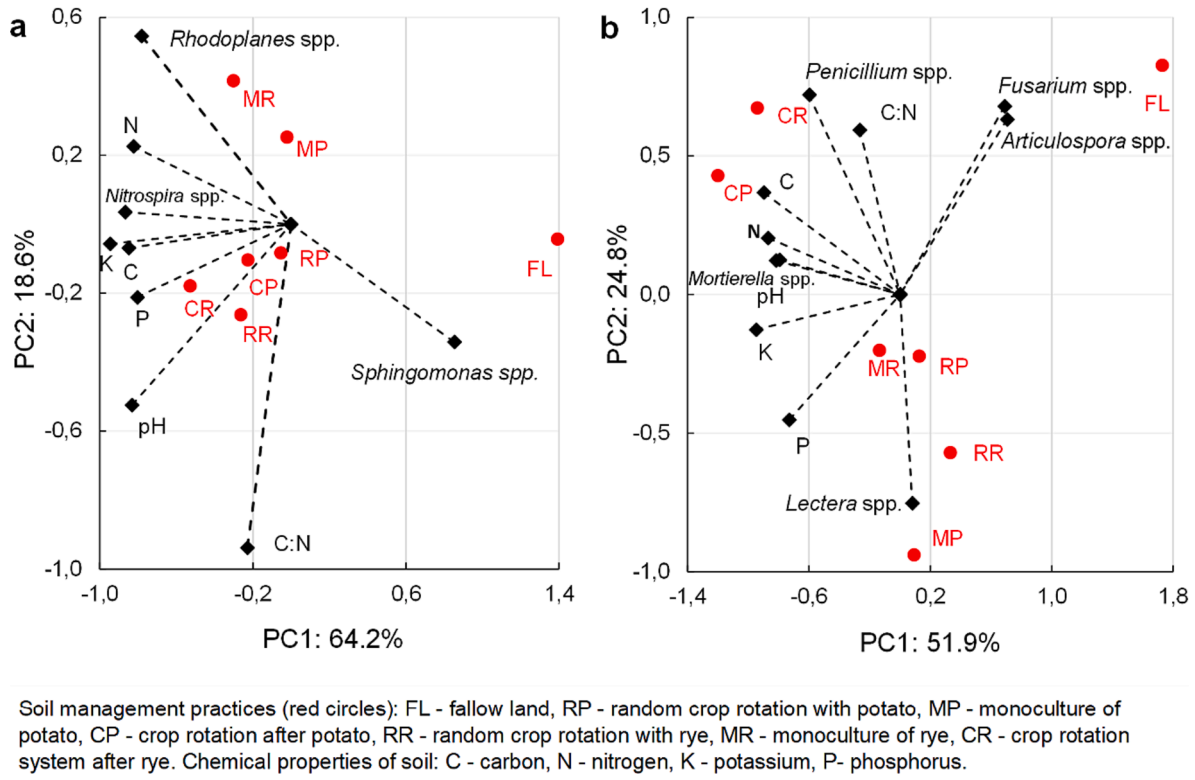


Fig. 5. Principal component analysis (PCA) of the abundance of the dominant bacterial taxa (a), dominant fungal taxa (b) at the genus level, and the chemical properties of soils from different agricultural practices.

Table 10
Dominant genera of fungi (relative abundance ≥ 1 % in at least one of the samples) in the experimental treatments (n = 3 per each soil sample).

Fungi/Genera	FL	RP	MP	CP	RR	MR	CR
Articulospora spp.	4,45b	0,00a	0,00a	0,00a	0,07a	0,02a	1,24a
Fusarium spp.	4,21b	1,20a	1,44a	1,28a	1,55a	1,87a	2,18a
Mortierella spp.	3,78a	15,35c	6,09a	16,74c	13,02c	12,74c	16,78c
Penicillium spp.	1,17b	1,33b	0,25a	3,08b	0,39a	0,29a	2,93b
Lectera spp.	0,08a	0,07a	4,42b	0,10a	1,44a	0,23a	0,15a

4.2.1. Structure of bacterial community

The dominant bacterial phyla in the agricultural soils include, among others, Proteobacteria, Actinobacteria, Acidobacteria, and Chloroflexi. As a rule, the relative abundance of these phyla is greater than 3 %, and, in the case of Proteobacteria, Actinobacteria, and Acidobacteria, often exceeds 10 % (Alami et al., 2021; Fierer et al., 2007; Janssen, 2006; Rao et al., 2021), which is in line with the results presented in our study. For instance, similar results were noted by Alami et al. (2021) who conducted a study on various monocultures, e.g. 2–year monoculture of sweet potato and 6–year maize monoculture (China); the relative abundances of dominants were: Proteobacteria – 33 %, Actinobacteria – 18 %, Acidobacteria –14 %, Chloroflexi – 13 %, Firmicutes – 3 %, Gemmatimonadota – 2 %, and Bacteroidota – 3 % (average values for all monocultures). Furthermore, in the soil from 10– year soybean and crop rotation, e.g. corn–soybean (Jilin Academy of Agricultural Sciences, China), the following relative abundances of dominant phyla were documented: Proteobacteria (approximately 24–42.9 %), Acidobacteria (approx. 12–25 %), and Actinobacteria (approx. 9–19 %) (Rao et al., 2021). The patterns above indicate that, despite the differences in crops and geographical location, the bacterial community (at the phylum or order level) in soil used for 100 years is relatively similar to that in soils used for a much shorter time.

Overall, these taxa of microorganisms play important functions in the soil environment. They are, among others, involved in the circulation of macro- and microelements, and in the transformation of organic matter and mineral compounds in soils. For instance, bacteria belonging to the phylum Proteobacteria are mostly copiotrophs, however, it is a diverse phylum where a lot of bacteria exhibit plant growth promoting traits including atmospheric nitrogen fixation, phytohormones production (e.g. indole-3-acetic acid, gibberellins, and cytokinins), and phosphorus solubilization (Dincă et al., 2022). Moreover, bacteria of the phylum Proteobacteria are capable of producing enzymes involved in the decomposition of organic matter, e.g. cellulases, xylanase, chitinase, glucanases, and proteases. Hence, the phylum Proteobacteria has a great importance for the carbon, nitrogen, and sulphur cycles in the soil (Ma et al., 2023; Spain et al., 2009). Therefore, referring to the fact that Proteobacteria (no statistical differences between FL and agriculturally used soils), Actinobacteria, or Gemmatimonadetes are mainly classified as copiotrophs, it can be concluded that the access to crop residues and fertilisation (resulting in a higher NPK content in agricultural used soils) increases their populations compared to FL. In our study, the patterns above are not entirely relevant in each of the treatments. Nevertheless, in terms of the phylum Actinobacteria, similar results were found by Alami et al. (2021); compared to the fallow land, the abundance of this phylum was higher in cultivations such as monoculture of maize (2 and 6 years) and *Fritillaria thunbergii* continuously cropped for 2 years. Interestingly, in the aforementioned study, similar to our case, the abundance of the phylum Proteobacteria was not

significantly lower in the soil from fallow land compared to agriculturally used soils. Importantly, in our study, more bacteria belonging to the phylum Nitrospirae were recorded in agriculturally used soils compared to the FL. This phenomenon can be explained by a higher nitrogen content in agriculturally used soils, resulting from fertilisation and crop residues. The availability of crop residues also stimulated the growth of the phylum Nitrospirae in the study by Li et al. (2020) who found a higher abundance of the phylum in tomato/potato-onion intercropping compared to monoculture. Interestingly, the highest abundance of the order WPS-2, which is also classified as copiotrophic bacteria (Bay et al., 2018), was noted in fallow land. According to Sheremet et al. (2020), the order WPS-2 is abundant in several dry, bare soil environments and thus probably could be adapted to FL soil.

In the study, there was a decrease in the abundance of Acidobacteria in most treatments compared to the FL. It can result from the economic life strategy of oligotrophic bacteria (Corrochano-Monsalve et al., 2021; Ho et al., 2017). Oligotrophic microorganisms are usually dominant in unfertilised soils, as confirmed by our study where their highest relative abundance was observed in the FL. Moreover, in the case of a lack of an available C source in the environment, oligotrophs can use weakly biodegradable compounds (e.g., humic compounds). Such a phenomenon can arise from the fact that unfertilised FL soil had the lowest content of biodegradable available C (Table 3). Furthermore, compared to the other variants, the highest abundance of Acidobacteria (statistically significant to most treatments) in the FL may also result from the low nitrogen content of the soil from FL. Interestingly, a significantly higher population of Acidobacteria was also detected in the soil from RP also characterised by a relatively lower nitrogen content compared to the other treatments (Table 3). Interestingly, the increased abundance of Acidobacteria in the FL and RP may also be linked to the low soil pH in these variants. Previously, similar patterns were also noted by Tayyab et al. (2021) who studied the bacterial community in a sugarcane monoculture. Moreover, these results are consistent with the study of Alami et al. (2021) who also reported the highest Acidobacteria population in soil from a fallow land compared to agriculturally managed soils – for instance monoculture of cabbage (2 years) and *Polygonum multiflorum* continuously cropped for 6 years. Additionally, similar patterns were noted in terms of the phyla Armatimonadetes and p_AD3, also mostly considered oligotrophic bacteria (Corrochano-Monsalve et al., 2021; Ye et al., 2021). Also autotrophic microorganisms such as Cyanobacteria or Planctomycetes (Fuerst, 2004) colonised FL most abundantly, which may result from the lower abundance of antagonistic copiotrophic bacteria. Interestingly, the above–mentioned members of oligotrophic bacteria were also negatively correlated with the abundance of copiotrophs of the phyla Actinobacteria and Gemmatimonadetes as well as with chemolithoautotrophic nitrificators belonging to Nitrospirae. Furthermore, in the case of the phylum Bacteroidetes, a higher abundance was also recorded in the fallow land compared to agriculturally managed soils. For instance, similar patterns were reported by Li et al. (2020) who also detected a lower abundance of Bacteroidetes in less nutrient-rich soil (tomato monoculture). Previously, similar results were also reported by Soman et al. (2017).

In terms of the order level, for instance taxa of the phyla Acidimicrobiales, Actinomycetales, Gaiellaes, or Solirubrobacterales (orders of the phylum Actinobacteria) were found in greater amounts in the agriculturally used soils compared to the fallow land, which, as in the case of the higher-ranking taxa (Actinobacteria), can arise from the fact that these orders are characterised by a copiotrophic lifestyle (Boubekri et al., 2022; Oh et al., 2012). Interestingly, a higher number of orders belonging to Proteobacteria, including Myxococcales, Rhizobiales, SC-I_94 (Betaproteobacteria class), and Xanthomonadales, were more abundant in the agriculturally used soils. The most abundant order in the studied soil was Xanthomonadales. Similar results were reported by Soman et al. (2017) who also conducted research based on long-term trials (the Morrow Plots, Illinois, USA). The authors noted a greater number of sequences belonging to this order in agriculturally managed soils e.g. in soil fertilised with manure (Soman et al., 2017). Therefore, in our case as well, the increase in the abundance of the order Xanthomonadales in the agriculturally used soils can be explained by a higher nutrient content of the soil. Interestingly, the order Xantomonadales includes numerous plant pathogens as well as beneficial bacteria including plant-growth promoting bacteria (Mansfield et al., 2012; Soman et al., 2017). Moreover, as in the case of the Morrow Plots experiments, we did not observe, for instance, changes in the quality of yields that could indicate plant infection. Hence, it can be theorised that these cultivation methods may have contributed to the growth of potential beneficial bacteria in the agriculturally used soils. Importantly, bacteria belonging to the order Myxococcales, which was more abundant in the agriculturally used soils, may improve soil structure by producing mucus, and produce antibiotics as well as numerous enzymes such as cellulases, chitinases, etc. (Wang et al., 2020).

In contrast, OTUs belonging to the orders Sphingomonadales, Rhodospirillales, and Burkholderiales were more abundant in the FL than in the agriculturally used soils. To our knowledge, currently there is no data in literature to confirm such a phenomenon. These bacteria colonise the rhizosphere and are included in the functional group of microorganisms promoting plant growth, including indicators of systemic resistance against e.g. *Fusarium oxysporum* (Jung et al., 2018). Also, the lower abundance of Roseiflexales (Chloroflexi) in the agriculturally used soils compared to the FL can arise from the fact that bacteria of this order are classified as oligotrophs (Xu et al., 2022).

Overall, the study results show that agricultural soil management may disturb the microbiological stability of the environment to a lesser or greater degree, as manifested in the change of proportions between the taxa of oligotrophic microorganisms dominant in the soil and copiotrophic microorganisms in comparison to FL (Fierer et al., 2007; Langer et al., 2004; Swędrzyńska and Grześ, 2015). Moreover, unlike several studies (Soman et al., 2017; Tayyab et al., 2021), our study did not report significant differences between bacterial dominant communities in monocultures and crop rotations, both at the phylum and order level. Interestingly, similar patterns were obtained by Alami et al. (2021). In our case, the phenomenon may be caused by favourable chemical properties of the soil from monoculture treatments. These chemical properties have a positive effect on the growth of bacterial populations.

4.2.2. *Structure of fungal community*

Three dominant phyla of fungi were detected in the studied soil – Ascomycota, Basidiomycota, and Zygomycota; these phyla were among the dominant fungi in other research works as well (Ai et al., 2018; Vanina G. Maguire et al., 2020). The highest abundance of the phylum Ascomycota was noted in the soil from the fallow land. It is probably due to the lack of fertilisers and pesticides and the cultivation of the soil for many years. For instance, different patterns were observed by Tayyab et al. (2021) who noted a high abundance of the Ascomycota in arable soil (sugarcane monoculture). The high abundance of this phylum in agriculturally used soils was also reported by Maguire et al. (2020) who conducted an experiment on rice monoculture and rice-pasture rotation systems (Argentina), and by Ai et al. (2018) who conducted a study on rice monoculture and rotation with rice (China). The discrepancies between the studies may be due to smaller amounts or a lack of fungicide application. However, these authors do not provide information on the use of such agents.

On one hand, the fungi abundance (mainly Ascomycota) is not conducive to the sanitary quality of the soil, as there are numerous pathogens and producers of numerous mycotoxins among the members of this phylum. However, numerous members of the phyla Ascomycota and Basidiomycota perform key functions in the soil environment. Namely, the involvement in biogeochemical cycles, the transformation and degradation of organic matter, the creation of humic substances, the mycorrhiza formation, and the plant growth promotion (Frąc et al., 2018).

In terms of the fungal taxa at the order level, the Pleosporales (class

Dothideomycetes) which includes phytopathogens (Egbuta et al., 2017; Gnavi et al., 2014; Ohm et al., 2012) dominated in all soils, and the largest number of its OTUs were found in the FL soil. The result could be influenced by the use of plant protection products in the agriculturally used soils. However, it is worth noting that the order Pleosporales includes not only phytopathogens but also saprophytes, epiphytes, and endophytes (Zhang et al., 2012).

While the OTUs belonging to Eurotiales (populating the CP and RR soil in large numbers) are mostly saprotrophs inhabiting mainly soil, some pathogens are also among them (Pangging et al., 2019). However, their increased abundance in these soils is not entirely clear. The highest abundance of the Mortierellales (the phylum Mucoromycota), which belongs to plant growth promoting fungi (PGPF), was noted in all variants with agricultural soil (Ozimek and Hanaka, 2021). The high abundance of this order in the agriculturally used soils may be explained by the presence of crop residues. For instance, similar patterns were found by Wang et al. (2017). Interestingly, the plant pathogens were also present among the fungi of the genus *Mortierella*, however very rarely (Eberl and Vandamme, 2016).

Moreover, the studied soils contained saprophytes applied in biocontrol, e.g. *Trichoderma* spp. usually colonising substrates rich in cellulose (Elad, 2000; Markovich and Kononova, 2003) and representatives of the Sordariales. The order includes ecologically diverse fungi e.g. like-mycorrhizal, endophytic, and rhizospheric fungi promoting plant growth, applied in biocontrol, and degrading organic residues and waste (Messiha et al., 2019; Zhang et al., 2006). Their relative abundance in the soil, even if fluctuating depending on the agricultural treatment, was lower or inconsiderably higher than in the FL. This pattern is not entirely clear; especially that according to Klaubauf et al. (2010), the dominant orders in agricultural soils are the orders Sordariales, Hypocreales, and Helotiales, which belong to the phylum Ascomycota.

4.2.3. Relationship between the relative abundance of potential microbiological health potential indicators and the soil properties of soil management practices

The abundance of *Nitrospir*a spp. depended on the soil management practice and a higher abundance was observed in the agriculturally used soils in comparison with the FL soil, which was certainly related to the highest nitrogen content, as well as organic carbon in these treatments. All species of the genus *Nitrospira* possess nitrite oxidoreductase genes and dominate the surface layer of the soil. Overall, *Nitrospira* spp. bacteria play an important role in the soil nitrification process and are dominant nitrifying bacteria in acidic soils with a pH < 6.17 (Hu et al., 2021). Importantly, due to the importance of soil nitrification processes, the promotion of *Nitrospira* spp. population in agriculturally used soils can be considered a very beneficial phenomenon, and can be considered a potential microbiological indicator of soil health.

The abundance of *Mortierella* spp. depended on the soil management practice. Hence, it affected the physicochemical properties of the soil, including C, N, and pH values. The highest content of C, N, and pH as well as the number of *Mortieriella* spp. were observed in the CP and CR; slightly lower values were obtained for the RR and RP. These patterns show that the order is associated with an increased and more diverse access to crop residues and decaying roots. The patterns above indicate that the rotation promotes an increased presence of the genus *Mortierella*, which is undoubtedly an added value due to the fact that a large number of fungi of the order are plant growth stimulants (Ozimek and Hanaka, 2021). Similarly, in podzolic and luvisol soil, where four crop species (potato-oat-barley-triticale) were grown in rotation, *Mortierella* spp. were found to dominate (Frąc et al., 2020).

5. Conclusions

In summary, the microbiota in agriculturally used soil is different from the microbiota of fallow land – both at the level of microbial diversity and the taxonomic composition of the microbiota. Importantly, the metagenomic analysis showed no significant differences between the taxonomic composition and the diversity of bacteria and fungi in different crop rotation and monoculture practices. Notably, the results of the study do not clearly show which taxa among bacteria and fungi can be used as a potential indicator of "soil fatigue". However, *Nitrospira* spp. (prokaryotes) and *Mortierella* spp. (eukaryotes) can be recommended as microbiological potential indicators for assessing "healthy agricultural soil" under the cultivation of potatoes and rye. Regarding the cultivation of the studied crops, the five–year crop rotation provides the best chemical and microbiological conditions of soil. Nevertheless, more extensive research is still needed to determine the microbiota in soils used for several decades or a century. In particular, RNA-seq studies should be carried out, allowing for a deeper analysis of the soil microbiota, which may contribute to the determination of new patterns shaping the relationship between long-term soil use and the soil microbiome.

CRediT authorship contribution statement

Ewa Beata Górska: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Writing – original draft. **Wojciech Stępień:** Validation, Project administration, Funding acquisition, Data curation, Conceptualization. **Edyta Hewelke:** Investigation, Formal analysis. **Jean-Christophe Lata:** Supervision, Resources, Methodology. **Barbara Gworek:** Writing – original draft, Project administration, Data curation. **Dariusz Gozdowski:** Visualization, Software, Formal analysis. **Lidia Sas-Paszt:** Validation, Investigation. **Stéphane Bazot:** Supervision, Resources, Methodology. **Anna Lisek:** Writing – original draft, Investigation. **Marcin Gradowski:** Writing – review & editing, Visualization, Software, Formal analysis. **Aneta Helena Baczewska-Dąbrowska:** Data curation, Software, Visualization, Writing – original draft, Writing – review & editing. **Jakub Dobrzyński:** Conceptualization, Investigation, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The research was partly financed by The Polish Ministry of Science and Higher Education (decision 8762E-385SPUB201). The next generation sequencing analysis was performed in Genomed S.A. (Warsaw, Poland) and we would like to thank Genomed Team for consultations.

The article was created thanks to cooperation of scientists from the Warsaw University of Life Sciences (Poland) and the Sorbonne University (France) beginning under the Erasmus+ program in 2019.

English language revision and translation assistance provided by Katarzyna Rafalska.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2024.111545.

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