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Original Article

Responses of soil physico-chemical properties, structure of the microbial community and crop yields to different fertilization practices in Russia's conventional farming system

Respostas das propriedades físico-químicas do solo, estrutura da comunidade microbiana e rendimento das culturas a diferentes práticas de fertilização no sistema agrícola convencional da Rússia

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Abstract

The use of fertilizers affects not only the soil fertility and crop yield, but also significantly changes the taxonomic structure of the soil microbiocenosis. Here, based on stationary field experiment, we studied the influence of organo-mineral fertilizer (OMF), modified by bacteria Bacillus subtilis, H-13 in comparison with different fertilizer systems (organic, mineral, organo-mineral) on (i) crop yield, (ii) physical and chemical properties, and (iii) alpha and beta diversity of the microbial community Albic Retisol (Loamic, Aric, Cutanic, Differentic, Ochric). The studies were carried out against the background of liming (pHKCl – 5.9) and without it (pHKCl – 5.1). The use of only one cattle farmyard manure was less effective than its co-application with mineral fertilizers in half doses. A similar effect was obtained when applying OMF. In addition, the use of OMF contributes to a significant increase in the reserves of soil organic carbon in the soil layer 0-20 cm by 18%-32%. Using high-throughput sequencing of the 16S rRNA variable V4 gene sequence libraries, 10.759 taxa from 456 genera were identified, assigned to 34 fila (31 bacterial and 3 archaeotic. Unilateral application of mineral fertilizers leads to a significant decrease in the alpha diversity of the structure of soil microbial communities (OTE (other things equal) and Shannon index). A clear clustering of the microbiota was found in the variants with and without the introduction of cattle farmyard manure. It is revealed that the taxonomic structure of the microbiocenosis is formed under the influence of two main factors: crop rotation culture and applied fertilizers. The type of cultivated crop determines the dynamics of the microbiota at the level of larger taxa, such as domains, and fertilizers affect the structure of the microbial community at a lower taxonomic level (phyla, orders, bloodlines). On the basis of the Deseq analysis, marker taxa were identified, according to the share participation of which it is possible to determine the type of cultivated crop and fertilizers used in the experiment. Understanding the dynamics of taxa association and other influential factors can lead to the creation of universal systems of metagenomic indication, where tracking the dynamics of microbial communities will allow for a comprehensive assessment of the agroecological state of soils and timely decisions to prevent their degradation.

Keywords: cattle farmyard manure, mineral fertilizers, liming, soil organic carbon, microbial community.

Resumo

O uso de fertilizantes afeta não apenas a fertilidade do solo e o rendimento das culturas, mas também altera significativamente a estrutura taxonômica da microbiocenose do solo. Aqui, com base em experimento de campo estacionário, estudou-se a influência do fertilizante organomineral (FOM), modificado pela bactéria Bacillus subtilis (H-13), em comparação com diferentes sistemas de fertilizantes (orgânico, mineral, organomineral) em (i) rendimento da colheita, (ii) propriedades físicas e químicas, e (iii) diversidade alfa e beta da comunidade microbiana Albic Retisol (Loamic, Aric, Cutanic, Differentic, Ochric). O experimento foi conduzido sob duas condições: com calagem (pHKCl - 5,9) e sem calagem (pHKCl - 5,1). O uso apenas de esterco bovino foi menos eficaz do que sua coaplicação com fertilizantes minerais em metade das doses. Um efeito semelhante foi obtido ao aplicar FOM. Além disso, o uso de FOM contribui para um aumento significativo nas reservas de carbono orgânico do solo na camada de solo de 0-20 cm em 18%-32%. O sequenciamento de alto rendimento das bibliotecas de sequências do gene variável V4 do rRNA 16S identificou 10.759 táxons pertencentes a 456 gêneros, classificados em 34 filos (31 bacterianos e 3 arqueanos). A aplicação unilateral de fertilizantes minerais levou a uma diminuição significativa da

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diversidade alfa da estrutura das comunidades microbianas do solo (OTE [outras coisas iguais] e índice de Shannon). Foi observada uma aglutinação da microbiota nas variantes com e sem a aplicação de esterco bovino. O estudo revelou que a estrutura taxonômica da microbiocenose é formada sob a influência de dois fatores principais: cultura de rotação de culturas e fertilizantes aplicados. O tipo de cultura cultivada determina a dinâmica do microbiota ao nível de taxa maiores, como os domínios, e os fertilizantes afetam a estrutura da comunidade microbiana a um nível taxonômico inferior (filos, ordens, famílias). Com base na análise Deseq, foram identificados taxa marcadores, de acordo com a participação dos quais é possível determinar o tipo de cultura cultivada e os fertilizantes utilizados na experiência. A compreensão da dinâmica da associação de taxa e de outros fatores influentes pode levar à criação de sistemas universais de indicaçadores metagenômicos, onde o rastreio da dinâmica das comunidades microbianas permitirá uma avaliação abrangente do estado agroecológico dos solos e decisões oportunas para prevenir a sua degradação.

Palavras-chave: esterco bovino, fertilizantes minerais, calagem, carbono orgânico do solo, comunidade microbiana.

1. Introduction

The sustainable development of agroecosystems is largely supported by a science-based fertilizer system (Edmeades, 2003). Numerous studies show that the use of organic, mineral, lime fertilizers and green manure can maintain soil fertility for a long time (Drinkwater et al., 1998; Zhao et al., 2009). Plowing of plant residues also preserves soil fertility (Blanchet et al., 2016). The physical, chemical, physico-chemical, and biological properties of soils largely determine the stability of agroecosystems (Doran, 2002; Nannipieri et al., 2003). An increase in the structure of sown areas of cereals and row crops with a reduction in cattle farmyard manure output makes it necessary to maintain SOC content at a certain level, which is determined by the type of soil, granulometric composition, specialization of crop rotation, and many other factors (Körschens et al., 2013). This problem is common in most countries of the world (Lützow et al., 2006; Rasmussen et al., 1998; Six et al., 2002). Organic and organo-mineral fertilization systems provide an additional supply of organic matter, thereby contributing to an increase in the content of SOC in the soil (Merzlaya, 2017, 2021).

The use of phosphorus fertilizers has a positive effect on the phosphate regime of the soil. Lukin (2009) noted an increase in the content of loosely bound phosphates, as well as aluminum phosphates, when applying cattle farmyard manure, compared to the mineral fertilizer system.

Most researchers are inclined to the need to determine the mobile forms of phosphorus and potassium in the soil not only in hydrochloric acid extract (0.2 M HCl), but also to determine the easily mobile forms in 0.01 M $CaCl_2$ (Houba et al., 1990; Kirpichnikov et al., 2003). When assessing the potash regime of soils, it is necessary to determine the so-called exchange potash in the 1M CH_3COONH_4 extract (Nikitina, 2018; Prokoshev and Deryugin, 2000).

Soil microorganisms are sensitive to climate and soil resource management practices. The taxonomic structure of soil microbial communities correlates with the beneficial functions of the soil and ecosystems, including nutrient cycling, neutralization of toxic compounds, and suppression of the development of harmful and pathogenic organisms. Based on the analysis of the soil microbiota, decisions can be made on the organization of land management to achieve maximum plant productivity (Galantini and Rosell, 2006).

A comprehensive analysis of the impact of various agricultural practices on the soil microbiota is possible only in the framework of stationary field experiments. For example, Wessén et al. (2010) showed that fertilization caused changes in basic parameters such as pH and carbon and nitrogen content in the soil. In their work, they studied the fertilization factor as the cause of changes in the composition of the microbial community.

Another study (Ramirez et al., 2010) showed a strong effect of the application of ammonium fertilizers on the biodiversity of bacteria. The effect of the ammonium nitrate dosage gradient on the functional profile of the community was shown using metagenomic indication (Fierer et al., 2012).

At the DOK station in Switzerland (Hartmann et al., 2015), where 5 farming regimes have been maintained since 1978, organic farming areas were characterized by an increased diversity of microbial communities. The application of organic fertilizers led to the accumulation of groups of copiotrophic microorganisms in the soil, while in areas with extensive agriculture, oligotrophic communities prevailed.

Some studies have shown the need for continuous application of fertilizers that increase the concentrations of silicon, total nitrogen, and other nutrients in the soil (Bi et al., 2009; Huang et al., 2010; Whitbread et al., 2003). The use of cattle farmyard manure significantly increased the content of carbon, total nitrogen, and other available nutrients and reduced soil acidification (Gu et al., 2009; Liu et al., 2011). Other studies have shown that continued use of fertilizers can lead to a decrease in soil quality and productivity (Kumar and Yadav, 2001; Yang et al., 2006).

Some studies (Sarathchandra et al., 2001) show that nitrogen and phosphorus fertilizers did not significantly affect soil microbial communities. While others (Belay et al., 2002; Chu et al., 2007; He et al., 2008; Zhong et al., 2007) have confirmed that mineral and organic fertilizers increase microbial biomass and microbial diversity.

Thus, there are quite contradictory data on the influence of agrochemical factors on the structure of the microbial community. That is why, using all the positive possibilities of long-term stationary experiment, we studied the influence of various fertilizer systems on the physical and chemical properties of the soil and the productivity of agricultural crops and determined the structure of the soil microbiocenosis, on the basis of which we identified marker taxa that are sensitive to the effects of various agrotechnical factors (fertilizers and crop rotation).

2. Materials and Methods

2.1. Site description and experimental design of filed experiment

The field experiment was laid in 2015 at the experimental field of Vereshchagin State Dairy farming Academy named after N.V. Vereshchagin in Vologda in a 5-field crop rotation: vetch-oat mixture for green mass; winter wheat-spring barley with sowing of meadow clover; meadow clover – oats. The experiment was developed on 3 consecutive input fields, which allowed to create a 3-fold repetition in time for each crop rotation.

The soil of the experimental field is sod-podzolic, light loamy according to the FAO classification-Albic Retisol (Loamic, Aric, Cutanic, Differentic, Ochric) characterized by 142 g $kg^{-1}$of clay, 213 g kg^{-1} of sand (WRB, 2015). The thickness of the arable horizon is 20 cm (Naliukhin et al., 2018a).

During the experimental period 2015-2020, mean annual rainfall and temperature were, respectively, 706 mm and 2.4 °C. During the warm period (April-October), 72% of the annual precipitation falls, during the cold period (November-March) – 28%. The scheme of the experiment included two factors (Table 1): A – liming (A1 – without applying $CaCO_3$ with an initial level of pH_{KCl} - 5.1 and A2 – applying $CaCO_3$ before creating pH_{KCl} - 5.9) and B – six fertilizer systems: (1) control (without fertilizers), (2) mineral (MIN), (3) organic (FYM) (cattle farmyard manure), (4) organo-mineral, (5) organo-mineral fertilizer (OMF) and (6) OMFb, the pellets of which are modified bisolbifit biopreparation (Naliukhin et al., 2018b).

Organo-mineral fertilizers are pellets and are made on the basis of lowland peat with the addition of macro-and micro-fertilizers, as well as humic acids. All fertilizer systems are balanced by the amount of nitrogen introduced, the dose of which corresponds to the intake of the element with cattle farmyard manure at a dose of 50 t ha^{-1} (N_{150}). Nitrogen doses were distributed according to the methodological recommendations for the north of the Non-Chernozem zone of Russia as follows: 30 kg N ha^{-1} was added to the vetch-oat mixture, 80-to winter wheat (N_{30} – in autumn and N_{50} – in spring for top dressing), 40 kg N ha^{-1} to barley with clover sowing (Sukov et al., 2018). The entire dose of cattle farmyard manure – 50 t ha^{-1} was applied to the vetch-oat mixture, phosphorus-potassium mineral fertilizers-to the first 3 crops of the crop rotation, and their aftereffect was studied on meadow clover and oats (Table 1). Composted cattle manure originating from loose housing was used as cattle farmyard manure, mineral fertilizers were applied in the form of a complex fertilizer – ammonium nitrate phosphate fertilizer, nitrogen – as ammonium nitrate (NH_4NO_3), potash – potassium chloride (KCl). The variants on each crop rotation field were placed systematically. The area of each plot was 50 m^2 (5 m x 10 m).

2.2. Soil sampling and agrochemical analyses

To determine the change in the agrochemical parameters of the soil at the end of the cycle crop rotation (in this article, the results are given for the 1st field), mixed samples were taken from the arable soil horizon (0-20 cm) in the autumn of 2020. Twenty cores with a diameter of 2.5-3.0 cm were randomly taken within each plot. Plant residues were removed from the soil, and individual samples were mixed to form a composite sample for each plot. The samples were dried in the oven at a temperature of 105 °C for 8 hours, sieved by 2 mm and analyzed for various soil properties (Table 2).

Agrochemical analysis of the soil was carried out according to the following methods: the acidity of the salt extract pH_{KCl} – potentiometrically (1 M KCl solution according to a soil: solution ratio of 1:2.5), hydrolytic acidity - according to Kappen in the modification of the Central Research Institute of Agrochemical Services for Agriculture (1 M CH_3COONa, ratio of 1:2.5), the amount of absorbed bases - according to Kappen-Gilkovitz (0.1 M HCl, ratio of 1:5) by titration (Mineev et al., 2001). Soil organic carbon content - according to Tyurin method in the modification of the Central Research Institute of Agrochemical Services for Agriculture (by oxidizing the organic matter with a bichromate mixture in a thermostat at t 110 °C and determining the optical density of the obtained solutions photometrically). Mobile forms of phosphorus and potassium-in the extract of 0.2 n. HCl (ratio of 1:5) according to Kirsanov in the modification of the Central Research Institute of Agrochemical Services for Agriculture based on All-Russian State Standard 26207-91 measured phosphorus mobility by a colorimetric method using a sulfomolybdic reagent. Potassium was determined in the resulting extract by the flame-photometric method. The readily mobile forms of phosphorus and potassium were determined according to Scofield (extracted with a 0.01 M $CaCl_2$ according to a soil: solution ratio of 1:20) (Houba et al., 1990). The subsequent analysis of the P_2O_5 content was carried out colorimetrically. In determining the exchange rate of potassium, the method used was according to Maslova (extracted with a 1M CH_3COONH_4 according to a soil: solution ratio of 1:20) (Prokoshev and Deryugin, 2000). Potassium in all extracts was determined by the flame-photometric method. The total nitrogen content in the soil was determined after wet salinization by Kjeldahl with further distillation of ammonia into boric acid, followed by titration; phosphorus and potassium-by the Ginzburg method, followed by determination of P_2O_5-by colorimetric method, K_2O-by flame-photometric method (Mineev et al., 2001).

Separately, soil samples were taken for subsequent laboratory analysis of the metagenomic structure by amplification and high-throughput sequencing of the 16S ribosomal RNA gene in real-time. Moist soil samples from the plough layer (0-20 cm) were collected for the estimation of microbial community structure. The samples were taken in the fall, immediately after harvesting the crop rotation and were frozen at -20 °C until laboratory analysis.

2.3. Soil microbial community structure

DNA was isolated from 0.5 g of frozen soil after mechanical destruction using zirconium beads in an extraction buffer consisting of the following components: 350 µl of solution A (sodium-phosphate buffer-200 mM;

Table 1. Applied mineral fertilization (kg ha^{-1}) and amendments (t ha^{-1}) on the crop rotation.

Crop	FYM			MIN			FYM + MIN			OMF			OMFb		
	N	P	K	N	P	K	N	P	K	N	P	K	N	P	K
Vetch-oats mixture	**50 t ha^{-1} manure application**			**30**	**30**	**30**	**25 t ha^{-1} manure application**			**30**	**30**	**30**	**30**	**30**	**30**
							15	**15**	**15**						
Winter wheat	0	0	0	80	30	60	40	15	80	80	30	60	80	30	60
Spring barley	0	0	0	40	60	135	20	30	67.5	40	60	135	40	60	135
Meadow clover	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oats	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total for crop rotation	150*	120*	225*	150	120	225	75 (150)**	60 (120)**	112.5 (225)**	150	120	225	150	120	225

*Nutrient uptake with 50 t ha^{-1} manure - composted cattle manure originating from loose housing; **Nutrient uptake with 25 t ha^{-1} manure and mineral fertilizers.

Table 2. Effect of the treatments on soil properties in the ploughed range of (0–20 cm) the year 2019-2020.

Crop	*FP:*	*Control*		*FYM*		*MIN*		*FYM + MIN*		*OMF*		*OMFb*		p-value		
	pH:	5.0	6.0	5.0	6.0	5.0	6.0	5.0	6.0	5.0	6.0	5.0	6.0	FP	pH	FP×pH
Organic properties																
SOC	[g.kg^{-1}]	1.54a	1.45a	1.71ab	1.52ab	1.48ab	1.46ab	1.74ab	1.58ab	1.78b	1.92b	1.86ab	1.72ab	0.027	n.s.	n.s.
C stock	[Mg.ha^{-1}]	38.5a	36.3a	42.8ab	38.0ab	37.0a	36.5a	43.5ab	39.5ab	44.5b	48.0b	46.5b	43.0b	0.029	n.s.	n.s.
Chemical properties																
pH	[KCl]	5.4aA	5.9aB	5.4 aA	5.8 aB	5.3 aA	5.8 aB	5.2 aA	5.9 aB	5.4 aA	5.8 aB	5.2 aA	5.9 aB	n.s.	<0.001	n.s.
H_r	[Cmol$_c$.kg^{-1}]	2.26aA	1.71aB	2.50 aA	1.69aB	2.86 aA	1.89aB	2.76aA	1.67aB	2.74aA	1.97aB	2.78aA	1.96 aB	0.042	<0.001	n.s.
S	[Cmol$_c$.kg^{-1}]	11.2aA	13.7aB	11.5 aA	12.8aB	10.5 aA	11.6aB	10.8aA	12.7aB	11.5aA	10.4aB	11.2aA	11.3 aB	n.s.	<0.001	n.s.
V	[%]	82.2aA	88.8aB	80.7 aA	88.4aB	78.4 aA	85.9aB	79.6aA	88.1aB	79.8aA	83.6aB	79.6aA	85.2 aB	n.s.	<0.001	n.s.
P_{HCl}	[mg.kg^{-1}]	249	272	244	259	250	272	260	273	280	266	276	215	n.s.	n.s.	n.s.
P_{CaCl2}	[mg.l^{-1}]	0.16	0.14	0.14	0.13	0.15	0.16	0.18	0.21	0.38	0.32	0.38	0.25	n.s.	n.s.	n.s.
K_{HCl}	[mg.kg^{-1}]	103 aA	86 aB	117 aA	89 aB	112 aA	93 aB	106 aA	105 aB	127 aA	97 aB	130 aA	103 aB	n.s.	0.001	n.s.
K_{AAE}	[mg.kg^{-1}]	116 aA	107 aB	126 aA	102 aB	126 aA	111 aB	114 aA	130 aB	164 aA	130 aB	161 aA	121 aB	n.s.	0.042	n.s.
K_{CaCl2}	[mg.l^{-1}]	14.0aA	13.0aB	10.0 aA	12.0aB	18.0 aA	14.0aB	11.0aA	10.0aB	18.0aA	12.0aB	19.0aA	17.0 aB	n.s.	0.015	n.s.

Displayed values are averages of the four replicates. P-values of each factor are computed according to an ANOVA. The abbreviation "n.s" stands for "not significant". Letters refer to the results of Tukey's HSD test and are only displayed if the significance threshold ($p < 0.05$) is reached. In addition, uppercase letters refer to the pairwise comparison of fertilization practices and lowercase letters to pH. FP: fertilization practice; pH: soil acidity level in treatments with or without liming.

guanidine isothiocyanate-240 mM; pH 7.0), 350 µl of solution B (Tris– HCl–500 mM; SDS–1% by weight to volume; pH 7.0) and 400 µl of a mixture of phenol and chloroform (1:1). The destruction of the sample was carried out for 1 minute at maximum power on the FastPrep 24 device ("MPMedicals", USA). The resulting preparation was centrifuged at maximum speed for 5 minutes. The water phase was sampled and re-extracted with chloroform. The DNA was precipitated by adding an equal volume of isopropyl alcohol. After centrifugation, the precipitate was washed with 70% ethanol and dissolved in water at 65 °C for 5-10 minutes. The DNA was purified by electrophoresis in a 1% agarose gel, followed by DNA extraction from the gel by sorption on silicon oxide (Chirak et al., 2013).

The pureness and amount of DNA in the preparation were determined by electrophoresis in 1% agarose in ½ TAE buffer (the average concentration of DNA in the sample was 50 ng/ml). Purified DNA preparations were used for quantitative PCR (qPCR) and the creation of amplicon libraries according to the instructions for the sequencing protocol ("Illumina, Inc.", USA). When creating libraries of 16S rRNA gene fragments for each soil DNA sample, PCR was performed with universal primers to the variable site V4 — F515 (5'-GTGCCAGCMGCCGCGGTAA-3') and R806 (5'-GGACTACVSGGGTATCTAAT-3') on a T100 thermal cycler (Bio-Rad, Germany) according to the following protocol: 3 minutes at 95 °C; 30 seconds at 95 °C, 30 seconds at 55 °C, 30 seconds at 72 °C (35 cycles). These primers are universal for prokaryotes. All primers contained service sequences with linkers and barcodes (required for sequencing using Illumina technology) (Evdokimova et al., 2020). Sequencing was carried out on the Illumina MiSeq device ("Illumina, Inc.", USA) in the Central Research Center "Genomic Technologies and Cell Biology" (All-Russian Scientific Research Institute of Agricultural Microbiology).

The raw data was preprocessed using the DADA2 package (Callahan et al., 2016) for the R programming language. The taxonomy was constructed using the DADA2 package, which provides a built-in implementation of the naive Bayesian classifier method for this purpose (Wang et al., 2007). The taxonomic composition in the samples was determined using the assign Taxonomy function.

Using the phyloseq package (McMurdie and Holmes, 2013) for the R programming language, eukaryotic taxa were removed from the samples. This software package is also used to further assess the similarity of communities.

The samples were grouped according to the principle of the plant cultivated on the corresponding accounting plots, and according to the fertilizers applied. For example, in the "Clover" group, all soil samples from the second field where meadow clover grew were collected, regardless of the fertilizers applied; similarly, in the "MIN" group, all soils that received only nitrogen-phosphorus-potassium fertilizers were collected. Thus, we have identified the following groups: "Clover" (C), "Barley" (B), "Oats" (O), "MIN", "OMF", "OMFb", "FYM + MIN", "FYM", "Control".

2.4. Data analyses

The grain yield was brought to the standard 14% humidity, the green mass of the vetch-oat mixture-to 80% humidity, followed by conversion to grain units (g.u.). Thus, results of grain units were expressed as a percentage of the control (without fertilizers) according to Equation 1 and were reported as relative grain yields.

$$\text{Relative yield} = \frac{\text{Absolute yield of a microplot, g.u.}}{\text{Absolute yield of a microplot in control, g.u.}} \times 100 \quad (1)$$

Statistical data processing was performed using the ANOVA two-factor analysis of variance model using the Statgraphics Centurion program. The analysis of differences between the variants was revealed using the criterion Tukey (Tukey test HSD), which allows us to more deeply identify the hidden interaction between the variants at $p < 0.05$ (Kiryushin et al., 2009).

To characterize the biodiversity and perform a comparative analysis of microbial communities, the parameters of alpha and beta diversity were calculated (Magarran, 1992; Whittaker, 1972).

Alpha diversity was evaluated using the Observed index (OTU number in the sample) and the Shannon index (Shannon Index, H) (Shannon, 1948). The significance of differences in alpha diversity indices between microbiomes was determined by the Kruskal-Wallis criterion. For pairwise comparison of samples, the Student's criterion (Student, 1908) was used.

To assess beta diversity, we used the UniFrac method (Lozupone and Knight, 2005), which allows us to identify the percentage of similarities between all pairs of compared microbiomes. UniFrac is a distance metric used to compare biological communities. It differs from other measures, such as the Bray-Curtis dissimilarity index, in that it includes information about the relative relatedness of community members by adding phylogenetic distances between observed organisms to the calculations. The unweighted (qualitative) UniFrac considers the presence or absence of observed organisms, while the weighted (quantitative) one considers their number. The distance was calculated between pairs of samples (each sample represented one community - in our case, one sample). All taxa found in one or both samples were placed on a phylogenetic tree. A clade (branch) leading to taxa from both samples was marked as "common", and branches leading to taxa that appear only in one sample were marked as "not common". Then the distance between the two samples was calculated as (sum of "non-common" branch lengths) / (sum of all tree branch lengths (= common + non-common)), i.e., the total fraction of branch lengths that are not common was determined. The data set was subjected to the procedure of combining repetitions and normalizing using a script *collapse_samples.py* embedded in QIIME (Caporaso et al., 2010).

Pairwise comparisons of the taxonomic composition of the samples were performed using the SEMPER test in the PAST4 program (Hammer et al., 2001).

SIMPER shows the species differences between the two groups using the Bray-Curtis index. The function determines the taxa that contributed the most to the group separation for each pair of comparisons in the beta diversity analysis.

The assessment of the influence of individual factors and their interaction on the taxonomic composition of the soil was carried out using permutation multivariate analysis of variance using distance matrices, Adonis. This analysis allows you to see whether the centroids of the beta variety are really different.

To identify specific marker taxa, Deseq analysis was used, which is an analysis of the differential representation of taxa based on a negative binomial distribution.

Deseq analysis, in comparison with SEMPER analysis, does not appeal to beta diversity and uses stricter criteria for differences in the representation of taxa for each pair of comparisons.

3. Results

3.1. Soil physico-chemical properties

The content of soil organic carbon (SOC) varied on average from 14.5 gkg^{-1} (Control-pH 6.0) to 19.2 $g.kg^{-1}$ (OMF-pH 6.0), which indicates a significant influence of the studied fertilizer systems ($p = 0.027$) (Table 2). Different levels of acidity did not have a significant effect on the change in the content of soil organic carbon ($p > 0.05$). Taking into account the volume weight of the soil in different versions of the stationary field experiment, C (carbon) stock was the greatest when using organo-mineral fertilizers-43.0-48.0 $Mg.ha^{-1}$. In comparison with the control (without fertilizers), there is a significant increase in the reserves of soil organic carbon in the arable soil layer by 18 and 32% when applying OMF and OMFb, properly (Table 2).

Liming significantly improves the physical and chemical properties of the soil (Table 2). Thus, the acidity of the salt extract (pH_{KCl}) significantly ($p < 0.001$) decreased from 5.0 to 5.8...5.9, hydrolytic acidity-from 1.78...2.05 (the initial level before the experiment was laid) to 1.67...1.96 mmol (eq)/100g, while the amount of absorbed bases in all variants of the experiment increased, as well as the degree of soil saturation with bases by 4...6 ($p < 0.001$). At the same time, the studied fertilizer systems do not have a significant effect ($p > 0.05$) on the physical and chemical properties of the soil (Table 2).

The use of fertilizer systems does not significantly affect the content of various forms of phosphorus and potassium in the soil ($p > 0.05$), extracted by various extracts, regardless of pH. At the same time, reducing the acidity of the soil from pH 5.0 to 5.8-5.9 reduces the mobility of potassium, reducing the proportion of K_{HCl}, K_{AAE}, K_{CaCl2} in comparison with non-limed soil. The content of various forms of phosphorus in the soil, however, does not change significantly ($p > 0.05$).

3.2. Crop yield

The relative yields of different crops during the entire crop rotation are reported in Table 3. All fertilizer system (FS) significantly affected the increase in the yield of all crops cultivated in the crop rotation ($p<0.001$). Liming increased the yield of the first 4 crops of the crop rotation, and the effect on oats was insignificant ($p > 0.05$). Two periods should be distinguished: the action and aftereffect of fertilizers. In the first period, the yield of vetch-oat mixture, winter wheat and barley in the variants with FP on the background of liming increased by 39-77% compared to the control (without fertilizers). In the second period (after the effect of fertilizers), the increase in the yield of clover and oats compared to the control was less and amounted to 22-31% and 17-25%, respectively. On average, during the cycle of the crop rotation, the highest yield was achieved with an organo-mineral fertilizer system against the background of liming (+43% to the control). Interaction of factors (FS and CaCO3) was not detected ($p> 0.05$).

3.3. Soil microbial community

The analysis of the taxonomic structure of the microbial community revealed 10.759 taxa from 456 genera assigned to 34 phyla (31 bacterial and 3 archaeotic. All soil samples are dominated by Actinobacteria, Actinobacteria, and Proteobacteria phyla, and archaeotic phyla are also present. The most numerous of the phylum archaea is Thaumarchaeota.

The evaluation of alpha diversity by the OTE number and the Shannon index (Figures 1 and 2) shows that unilateral application of mineral fertilizers contributes to a significant decrease in the structure of soil microbial communities. Only on the last crop of the crop rotation-oats, there is a smoothing of the differences between the variants.

When assessing the beta diversity, the clearest clustering was found between the control variant (without fertilizers) and the variant with cattle farmyard manure (Figure 3).

The Adonis analysis showed significant differences ($p < 0.05$) in the influence of each of the studied factors (FS and $CaCO_3$) separately and their interactions on the taxonomic composition of the soil microbiota (Table 4). The calcification factor ($R^2 = 0.011$) makes an unreliable contribution to the beta diversity of soil microbial communities according to the results of the analysis.

The most powerful influence on the structure of the microbiome is the interaction of fertilizer systems and crops cultivated in the crop rotation ($R^2 = 0.116$). At the same time, other factors have the greatest influence on the formation of the taxonomic composition ($R^2 = 0.578$). In this case, it seems that we are talking about the "common community", which is the most conservative and the least susceptible to change.

4. Discussion

4.1. Influence of fertilization practices on soil physico-chemical properties

The use of all fertilizer systems, with the exception of MIN, contributed to the preservation of the initial SOC content (15.7 gkg^{-1}). The addition of OMF increased the soil carbon content from 13 to 22% and from 17.8 to 19.2 gkg^{-1} (Table 2). On average, organic carbon reserves in the arable (0-20 cm) soil layer increased by the same amount. Liming in the first years contributed to the increased mineralization of SOC, and in subsequent years

Table 3. Average yield per crop type.

Crop	*FP:*	*Without fertilizer (Control)*		*FYM*		*MIN*		*FYM + MIN*		*OMF*		*OMFb*		p-value		
	pH:	5.0	6.0	5.0	6.0	5.0	6.0	5.0	6.0	5.0	6.0	5.0	6.0	FP	pH	FP×pH
Vetch-oats mixture 2015-2017		100aA	114.15aB	127.08bA	142.77bB	133.23bA	149.23bB	142.77bA	159.08bB	136.31bA	150.77bB	145.23bA	166.77bB	<0.001	<0.001	n.s.
Winter wheat 2016-2018		100aA	111.88aB	128.35bA	148.66bB	146.36cA	168.20cB	154.02cA	177.01cB	148.28cA	167.43cB	157.85cA	175.86cB	<0.001	<0.001	n.s.
Spring barley 2017-2019		100aA	118.33aB	116.67aA	138.89aB	147.78bA	170.00bB	151.11bA	171.67bB	156.11bA	177.22bB	169.44bA	178.89bB	<0.001	<0.001	n.s.
Meadow clover 2018-2019		100aA	104.35aB	112.42bA	122.02bB	111.27bA	123.56bB	114.98bA	131.63bB	117.80bA	127.27bB	118.69bA	127.66bB	<0.001	<0.001	n.s.
Oats 2019		100a	107.87a	109.15ab	119.79ab	112.55ab	117.23ab	125.53b	122.77b	114.26a	110.43a	115.11 ab	115.96ab	<0.001	n.s.	n.s.
Average 2015-2019		100aA	109.18aB	116.63aA	130.02aB	123.08bA	136.23bB	130.27bA	143.67bB	127.54bA	136.97bB	131.76bA	133.00bB	<0.001	<0.010	n.s.

Displayed values are averages of the four replicates. P-values of each factor are computed according to an ANOVA. The abbreviation "n.s" stands for "not significant". Letters refer to the results of Tukey's HSD test and are only displayed if the significance threshold ($p < 0.05$) is reached. In addition, uppercase letters refer to the pairwise comparison of fertilization practices and lowercase letters to pH. FP: fertilization practice; pH: soil acidity level in treatments with or without liming. Values with different letters (a–d) within a row indicate significant differences between treatments ($p < 0.05$).

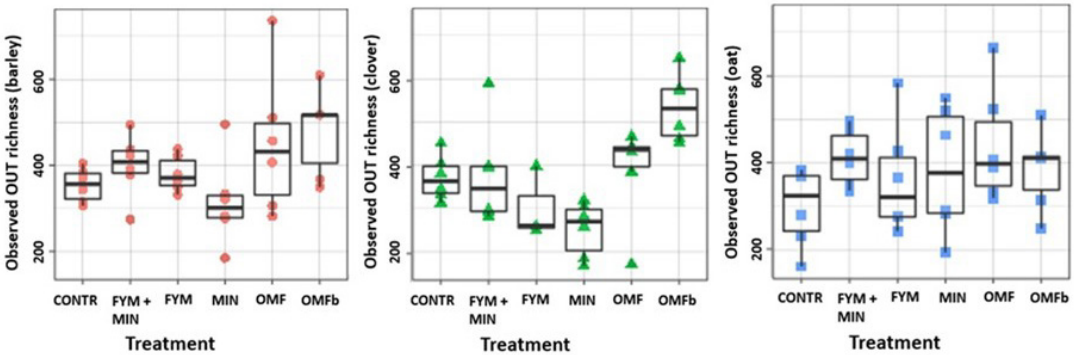

Figure 1. Boxplots of taxonomic diversity of microbial communities based on observable operating taxonomic units (OTUs) and Shannon indices for alpha diversity in the ploughed range of (0-20 cm) for the different treatments. Boxes are median and 25th and 75th percentiles. Whiskers are non-outlier ranges.
Symbols: CONTR-control, FYM-manure dosage of 50 t ha^{-1}, MIN - NPK-nitrogen-phosphorus-potassium fertilizer, FYM+MIN-manure dosage of 25 t ha^{-1} + NPK-nitrogen-phosphorus-potassium fertilizer, OMF-organo-mineral fertilizer, OMF+B-organo-mineral fertilizer with the addition of the biological product Bisolbifit.

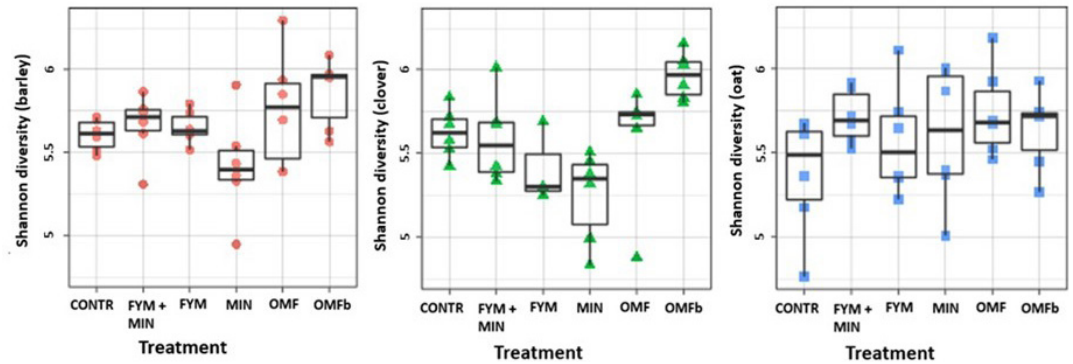

Figure 2. Boxplots of taxonomic diversity of microbial communities based on Shannon indices for alpha diversity in the plough layer (0-20 cm) for the different treatments. Boxes are median and 25th and 75th percentiles. Whiskers are non-outlier ranges.

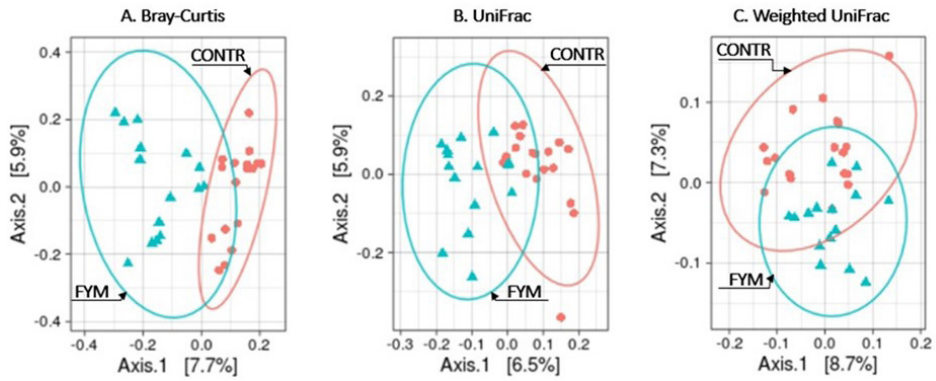

Figure 3. Graphs of beta diversity from samples from the control field (red dots) and manure fertilized at a dose of 50 t ha^{-1} (blue dots), using three different similarity metrics (Bray - Bray-Curtis metric; unifrac - unweighted (qualitative) UniFrac; wunifrac - weighted (quantitative) UniFrac).

there was an increase in the content of organic carbon due to a greater accumulation of plant-based root residues into the soil with their subsequent humification.

After the completion of the 5-year rotation, there is a tendency to reduce the content and reserves of SOC in the control (without fertilizers) and in the mineral

Table 4. Assessment of the influence of individual factors and their interaction on the taxonomic composition of the soil microbiota according to the adonis2 statistical analysis.

Factor	Degrees of freedom	Sum of squares	R^2	F	Pr (> F)
Fertilizer	5	3.033	0.091	2.162	0.001
Crop	2	1.418	0.042	2.526	0.001
Liming	1	0.364	0.011	1.297	0.014
Fertilizer: Crop	10	3.885	0.116	1.384	0.001
Fertilizer: Liming	5	1.657	0.049	1.181	0.001
Crop: Liming	2	0.666	0.020	1.186	0.023
Fertilizer: Crop: Liming	10	3.095	0.092	1.103	0.003
Residual factors	69	19.363	0.578	-	-
Total	104	33.481	1.000	-	-

fertilizer system (Table 2). Apparently, the plowing of plant residues alone cannot stabilize the humus state of soils (Anisimova et al., 2019). The introduction of FYM at the rate of 10 tons ha^{-1} $year^{-1}$ contributes to the preservation of the initial content of organic carbon in the soil. According to our research, such a dose of cattle farmyard manure stabilizes the humus state of soils in field crop rotations in the Non-Chernozem zone of Russia (Sychev et al., 2020).

Liming previously slightly acidic soil increased the pH of the experimental variants to neutral (pH 5.8-5.9), significantly reduced the value of hydrolytic acidity and increased the amount of absorbed bases ($p < 0.001$). The degree of soil saturation with bases significantly increased (83.6-88.8%).

At the same time, the use of fertilizer systems does not significantly affect the phosphorus content extracted by the Kirsanov hydrochloric acid extract and the Scofield $CaCl_2$ extract ($p < 0.05$) (Table 2). This is most likely due to the initially high content of mobile phosphorus in the soil (260-270 mg kg^{-1} according to the Kirsanov method), which is associated with a high content of phosphorus in the parental soil-forming rocks.

To study the effect of fertilizer systems on the potash regime of the soil, 3 forms of K_2O extracted by various extracts were determined: mobile potassium according to Kirsanov, exchange potassium according to Maslova, and light–mobile potassium according to Schofield (Table 2). As a result of the dispersion analysis, it was found that the content of various forms of potassium significantly depended only on the acidity of the soil ($p<0.05$), and fertilizer systems did not have a significant effect ($p>0.05$). There was also no conjugation effect from the interaction of factors (Table 2) Cultivation of crops without fertilizers (Control) led to a negative balance of potassium, as a result of which the content of mobile K_2O decreased by 20%, exchange-by 35% and light-mobile by 60%, compared to the initial level. At the same time, all fertilizer systems contributed to an insignificant increase in the content of mobile potassium. When analyzing soil samples using the Maslova method, the extraction of 1M CH_3COONH_4 was approximately 10...12% more K_2O than in 0.2 M HCl-extraction according to Kirsanov, which is associated with the most complete displacement of exchangeable potassium from the soil. The definition of light-mobile potassium also reflects the identified patterns.

4.2. Long-term effects on crop yield

During the rotation, crop yield was influenced by both fertilization practices (FYM, MIN, FYM+MIN, OMF and OMFb) and $CaCO_3$ application (Table 3). It should be noted that the cattle farmyard manure application contributed to an increase in crop productivity, both in the year of action and during the 3 years of aftereffect. The use of manure in conjunction with $CaCO_3$ contributed to an additional yield (on average per rotation of the crop rotation) of 12% in relation to unlimed soil. The advantage of the MIN fertilizer system over FYM was shown on cereals: winter wheat and spring barley against the background of liming, where an additional increase of 13% and 23%, properly, was obtained (Table 3). Organo-mineral fertilizer system is traditionally considered the best for the zone of sod-podzolic soils. In our experiment, it also showed its advantage over other fertilization practices, especially in the aftereffect on oats. The combination of organic fertilizers with mineral fertilizers contributed to an increase in the use of nutrients from these fertilizers (Aliev et al., 2016). The study of new types of organo-mineral fertilizers (OMF) showed some advantage over mineral fertilizers (MIN). A particularly strong effect was observed from OMFb, the granules of which were modified with the Bisolbifit biological product. The yield increase from OMFb on an unlimed background at pH 5.1-5.2 was 6.5% to MIN at the same acidity, which is consistent with previous studies (Naliukhin et al., 2018c).

Liming improved the physical and chemical properties of the soil (Table 3) and, as a result, increased the return on fertilizers. The most responsive crop for liming was spring barley (Table 3). The yield of barley on various fertilizer systems from the application of $CaCO_3$ increased by 14-15%. Meadow clover, on average, increased the yield from liming by 4-11% (Table 3). Oats, as a culture resistant to the acid reaction of the universe, turned out to be weakly sensitive to liming ($p > 0.05$). In general,

it should be noted that liming even slightly acidic soil contributes to a significant increase in crop productivity and increases the efficiency of various fertilizer systems (Naliukhin et al., 2017).

4.3. Influence of fertilization management on soil microbial community

The soil of the experiment is dominated by the phylum Actinobacteria, Acidobacteria, and Proteobacteria, and the archaeotic phylum is also present. The most numerous of the phylum archaea is Thaumarchaeota. In a crop rotation field sown with clover and fertilized with cattle farmyard manure at a dose of 50 t/ha, actinobacteria becomes more abundant than in fields with barley and oats. Also, these fields lack the phylum Bacteroidetes and Planctomycetes. In the variants with the introduction of manure, regardless of the host plant, OTE from the Actinobacteria phylum predominate and the relative number of microorganisms belonging to the Verrucomicrobia phylum decreases, compared to other crops. The number of Bacteroidetes increases in the fields under meadow clover and a mixture of barley and clover when using organo-mineral fertilizers with the addition of Bisolbiphite. In an earlier study, the dominant phylums under the vetch-oat mixture were identified, the proportion of which was significantly higher than the rest. These include: Actinobacteria (7.1-11.5%), Actinobacteria (13.6– 20.4%), Bacteroidetes (7.2–19.3%), Proteobacteria (45.3–56.2%), Verrucomicrobia (4.3–10.3%). The highest proportion of Actinobacteria (more than 20%) was observed in the variants with the introduction of OMF and OMFb with bisolbiphite, which indicates that the use of these fertilizers has a positive effect on their development (Naliukhin et al., 2018a).

4.3.1. Alpha diversity of soil microbial communities

In the present study, alpha diversity was evaluated using the Observed index for the number of OTE in soil samples and the Shannon index. The significance of differences in alpha-diversity indices between the microbiome in different variants of the field experiment was determined by the Kruskal-Wallis criterion. For the pairwise comparison of the variants, the Student's criterion was used.

The research results show that the unilateral use of mineral fertilizers (NPK variant) significantly reduces the diversity of soil microbial communities, regardless of the crop cultivated in the crop rotation (Figures 1 and 2).

At the same time, the co-application of NPK with manure, and especially the use of organo-mineral fertilizers (OMF), contributes to an increase in the level of species richness and diversity (Figures 1 and 2). Thus, it can be assumed that the use of OMF creates a high functional stability of the soil microbiome, allowing it to quickly recover during negative impacts (Nannipieri et al., 2003; Yin et al., 2000). This was confirmed by a pairwise comparison of soil microbial communities according to the Student's criterion for the OTE and Shannon indices.

The taxonomic structure of the soil microbial community significantly depends on the timing of fertilizer application and the cultivated crop. The most variable microbiocenosis of the soil is in the cultivation of barley, under which increased doses of phosphorus-potassium fertilizers were applied, as well as in the 1st year of the aftereffect of fertilizers on meadow clover (Figures 1 and 2).

In the 2nd year of the aftereffect of fertilizers on oats, the alpha-diversity indices for the experimental variants differ the least (Figures 1 and 2). This indicates the alignment of the structure and diversity of microbial communities after the termination of fertilization between the variants of the experiment. Also, in our opinion, oats, a crop with a strong root system and a phytosanitary effect on the soil, have a great influence on smoothing out differences in the microbiome.

4.3.2. Beta diversity of soil microbial communities

Beta-diversity assessment using three different similarity metrics (Bray-Curtis, unweighted (qualitative) and weighted (quantitative) UniFrac) revealed clustering of the control microbiomes (without fertilizers) and the 50 t ha^{-1} manure variant (Figure 3). Clustering was less pronounced between the other variants. Further analysis was carried out to understand the influence of which taxa makes the greatest contribution to the division of communities.

4.3.3. Marker taxa of soil microbial communities - indicators of the types of fertilizers and crops used

Despite the decrease in soil acidity pH_{KCl} from 5.1-5.2 to 5.8-5.9, the change in the proportion of such phyla as Proteobacteria, Cyanobacteria, Acidobacteria, Actinobacteria was insignificant (Table 4). Based on the results of the Adonis and Deseq tests, it can be concluded that the liming factor does not significantly affect the change in the taxonomic composition of the microbiome (in this given pH range).

The analysis of the data obtained using the methods of multivariate statistics shows that the taxonomic structure of the microbiocenosis is formed under the influence of two main factors: crop rotation culture and applied fertilizers. The type of cultivated crop determines the dynamics of the microbiota at the level of larger taxa, such as domains, and fertilizers affect the structure of the microbial community at a lower taxonomic level (phyla, orders, bloodlines).

It was found that the Semper analysis, unlike Deseq, is not specific for each factor affecting the soil microbiome. The same taxa are defined for most factors. That is why in this paper we present the Deseq-defined marker taxa of the soil microbial community, which has a specific reaction to the studied crops and fertilizers (Table 5).

These include 8 phylas, which is 2 times more than Simper determined (4 phylas). The phylas Proteobacteria, Firmicutes, Actinobacteria, Chloroflexi, and Acidobacteria are represented by several bloodlines. Single bloodlines include the phylas Gemmatimonadetes, Thaumarchaeota, and Verrucomicrobia.

Thus, in our sample of data with a small number of repetitions Deseq analysis is best for identifying a series of specific marker taxa.

Table 5. Marker taxa based on the results of the Deseq analysis for all pairs of comparisons.

Fila	Kind	O/C	O/B	C/B	MIN	FYM+MIN	FYM	OMF	OMFb
Acidobacteria	Pyrinomonadaceae			+					
Acidobacteria	NA								
Verrucomicrobia	Chthoniobacteraceae						+		
Actinobacteria	NA	+	+				+		
Actinobacteria	Gaiellaceae					+			+
Actinobacteria	67-14								+
Actinobacteria	Solirubrobacteraceae								+
Actinobacteria	Sporichthyaceae				+				
Actinobacteria	Nocardioidaceae						+		
Chloroflexi	JG30-KF-CM45				+				
Chloroflexi	NA						+	+	+
Firmicutes	Planococcaceae						+		
Firmicutes	Clostridiaceae_1						+		
Proteobacteria	Xanthobacteraceae							+	
Proteobacteria	Mitochondria				+				
Proteobacteria	A21b						+		
Gemmatimonadetes	Gemmatimonadaceae			+					
Thaumarchaeota	Nitrososphaeraceae			+			+		

O = oats; B = barley; C = clover. Samples with different types of applied fertilizers were compared with the control.

5. Conclusions

In general, biomodified organo-mineral fertilizers (OMF) had the same effect on soil properties as the co-application of cattle farmyard manure (5t ha^{-1} $year^{-1}$) with mineral fertilizers. The OMF application keeps the soil organic carbon content at the original level that it was before the experience establishment. Liming significantly reduced the acidity of the soil and increased the efficiency of all the studied fertilizer systems. There is a pattern of decrease in the content of mobile forms of potassium during neutralization of soil acidity, which is largely due to the antagonism between potassium and calcium in the soil-absorbing complex of sod-podzolic light loamy soil. The use of mineral fertilizers alone leads to a decrease in the taxonomic diversity of the soil microbial community, while the use of manure, as well as the joint application of manure with mineral fertilizers, contributes to an increase in the level of species richness and diversity of the soil microbiome. It was found that when applying fertilizers, taking into account the specific characteristics of crops, special specific microbial communities are formed, among which it is possible to distinguish marker taxa, which can (be used to) determine the direction of soil processes and make timely decisions to prevent soil degradation.

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