Comparative investigation of a microbial biodiversity in agroecosystem on grey forest soil at application of manure and mineral fertilizers

Seliverstova O.[1], Verkhovtseva N.[1], Kirushin E.[1], Korchagin A.[2] ,Osipov, G.[3]
[1] Agrochemistry, Faculty of Soil Science, Moscow Lomonosov State University, Russia seliv78@list.ru
[2] Scientific Research Institute of Agriculture, Vladimir region, Suzdal, Russia
[3]Research group of Academician Ju.Isakov, Russian Academy of Medical Sciences, Moscow, Russia

Introduction

It is known that agrochemical agents usually increase the content of organic matter (C_{org}) in soil, in which case the numbers of microorganisms, as rule, also increase [1-3]. The use of only organic fertilizer, however, can lead to rapid mineralization of both applied (allochthonous) and own (autochthonous) organic matter as a result of disturbing metabiosis – the interrelation of food chains of processing nutrients by individual organisms in soil [4]. The use of only mineral fertilizers can cause a decrease of the biomass of microorganisms in soil [5]. The combined application of these fertilizers provides a positive balance of C_{org}, reduces its mobility and increases the proportion of high-molecular-weight components; however, it sometimes leads to loss of humus by soil due to intensive mineralization [6]. Consequently, data not only on total numbers of microorganisms but also on the structure of the soil's microbial community, notions about which are broadening in connection with the use of molecular methods of investigating their microbocenoses, are important [7-9]. At studying the soil populations of the microorganisms which cannot be or difficultly cultured by standard laboratory practices or what cannot be grown up *in vitro* without obligate symbiosis as in case of arbuscular mycorrhizal fungi, use of molecular methods is represented actual.

The purpose of the work was assess the effect of organic and mineral fertilizers, a species and a phase of culture's development of a crop rotation and climatic condition on a change in the microbial community both qualitatively and quantitatively. The composition of microbial communities was investigated by gas chromatography-mass spectrometric (GC-MS) analysis of chemical signature markers without the use of any conventional cultural techniques.

Materials and methods

The surface soil samples (0-20 cm depth) were taken in May and August 2002 and 2003 from plots of a long-term field experiment. The experiment was initiated on grey forest soil (Vladimir region, Russia) in 1997. The scheme of experience included four variants: 1 - zero, 2 - supporting 3 and 4 - intensive. In 2002 were inputs according to variants of experience 1–cattle manure 40 t, 2-cattle manure 40 t + N30, 3- cattle manure 40 t + N60 and 4- N120P60K60. In 2003 –1 - fertilizers were not applied in variant, 2 - N30P50K90, 3 - N30P60K120 and 4 - N140P50K190. Chemical fertilizers were applied in the form of NH_4NO_3, $Ca(H_2PO_4)_2$ and KCL. Six-course a crop rotation was applied. 1 – winter wheat, 2, 3 - winter rye and 4 - spring wheat was grown in 2002, respectively to variants. In 2003 on variants 1-3 was grown oats, on 4 - potato. Soil properties were determined using standard methods [10]: measurement of C_{org} by rapid-response analyzer AN-7529, available P – Kirsanov technique, NH_4^+ - with Nessler reagent measurement and NO_3^- - water extract. The composition of bacterial communities, fungi and Arbuscular mycorrhizal (AM) fungi was defined by the method of gas chromatography - mass spectrometry (Agillent Technologies, USA) by using specific chemical markers of walls of microbial cells [11].

Results and discussion

Values pH of investigated samples statistically did not differ from size of this parameter in initial soil which amounts 6,8. Application of manure in 2002 led to decrease in this parameter in spring period. In the content of organic carbon at comparison of variants of a crop rotation fluctuations were observed. The content of mobile phosphorus in soil is an average, of exchange potassium - high. The content of NO_3^- in soil is low (Table 1).

By the method of GC-MS it is shown, that the community of microorganisms of grey forest soil includes 50 - 45 species (37 - 33 genera), in 2002 and 2003, accordingly. The dominating species (microorganisms, whose relative abundance exceeded 10 %), in rhizosphere of cereal cultures in 2002 were *Arthrobacter globiformis* which have ubiquitous presence in soil and also actinomycetes *Micromonospora* sp., *Rhodococcus equi*, *Nocardia asteroids*, *N. carnea*, which are capable to produce depolymerization enzymes for recalcitrant compounds. In 2003 dominant species were *Acetobacter diazotrophicus*, *Aeromonas hydrophila, Clostridium pasterianum, Butyrivibrio* spp., *Propionibacterium freudenreichii, Rhodococcus equi*. It is necessary to note, that meteorological conditions of studied years essentially differed counting upon hydrothermal factor (HTF) [12]: 2002 was characterized by insufficient humidification (HTF =0,85), and 2003 – by increased humidification (HTF =1,97). Thus, in 2002 there were favorable conditions for development of mainly aerobic microorganisms whereas at the increased humidification of soil among of the dominating species have noted been anaerobic and facultatively-anaerobic bacteria. Number of microorganisms by the end of vegetation of 2002 has increased in all investigated samples, having the greatest sizes in a variant with application of full mineral fertilizer (Table 2), that testifies to absence of a competition between plants and microorganisms for accessible substances and additional supply of microorganisms by substances from rhizodeposition. In 2003 the total number of bacteria was above, than in 2002, but the return tendency was observed: during the autumn period the total number of microorganisms has decreased, except for a variant with application of N30P60K120. Apparently, the limiting factor was low security of the soil by nitrate (Table 1). Changes in number of separate groups of microorganisms were reflected in structure of microbocenoses. So, during active growth of plants in 2002 two ecologo-trophical groups of microorganisms - actinomycetes and coryneform bacteria dominated. In 2003 hydrolytic and fermenting bacteria has essentially increased in the general block of microorganisms due to decrease, mainly, of actinomycetes. Contents C_{org} in the soil for the vegetative period at cultivation of an oats in variant without application of mineral fertilizers (after-action manure) and at application of full mineral fertilizer did not decrease, of ammonium – was increased. Cultivation of a potato on a background of application of mineral fertilizer in dose N140P50K190 in these climatic conditions led to decrease C_{org} and ammonium nitrogen. In the given variant the narrowest ratio C:P which characterizes a degree of susceptibility of organic substance of soil to microbiological decomposition [13] is noted. Thus, cultivation of a potato at the increased humidifying during the vegetative period promoted high intensity of processes of a mineralization of organic substance of the soil.

Application of cattle manure in 2002, according to Shannon diversity indices, promotes increase in quantity of microbial species in early terms of plant vegetation. A microbial biodiversity by the end of vegetation was decreased in these variants. The estimation of a microbial diversity of the soil taken for the analysis in 2003, has shown, that in variants under crop of an oats (variants 1-3) Shannon diversity indices increased by the end of vegetation. Application of full mineral fertilizer in dose N140P50K190 (a variant with cultivation of a potato) has led to decrease in a biodiversity of microorganisms by the end of the vegetative period, but came nearer to values of diversity indexes at cultivation of cereal cultures in the previous rotation.

The analysis of data AM fungal signature markers (16:1ω5) and other fungal signature markers by the method of GC-MS has shown (Table 2), that the content of microscopic

fungi depends on temperature and humidity. So, the dry vegetative period of 2002 has led to decrease in a biomass of fungi by the end of vegetation of plants. The high humidity of the vegetative period of 2003 has promoted growth of fungus in the soil. Besides it is noted, that the diversity index has positive correlation with the contents in the soil of fungus that testifies to stimulating influence of the index on a biodiversity of microorganisms of grey forest soil.

Table 1. Agrochemical characteristics of grey forest soil at application of organic and mineral fertilizers in 2002/2003

Variants[a]	pH_{H_2O}	C_{org} %	P_2O_5 mg/100 g	NH_4^+	NO_3^-
May					
1	6,1 / 6,6	1,6 / 1,9	8,8 / 8,03	3,4 / 4,9	0,7 / 1,0
2	6,1 / 6,5	2,0 / 1,9	9,5 / 15,2	4,8 / 4,2	0,7 / 1,0
3	6,7 / 6,7	1,8 / 1,2	15,0 / 12,6	5,6 / 4,0	0,8 / 1,1
4	6,3 / 6,5	1,8 / 1,1	12,5 / 14,4	6,2 / 3,1	0,8 / 1,2
August					
1	6,6 / 6,7	1,5 / 2,0	10,5 / 10,7	2,0 / 4,7	1,0 / 0,8
2	6,2 / 7,2	2,0 / 1,6	9,5 / 9,2	2,9 / 4,5	0,8 / 0,6
3	6,5 / 7,0	1,6 / 1,5	11,5 / 8,2	3,8 / 4,1	0,8 / 0,8
4	6,9 / 6,8	1,8 / 1,2	15,9 / 10,5	3,3 / 3,4	1,1 / 1,2
± SE[b]	0,2 / 0,2	0,05 / 0,05	2,3 / 2,5	0,21 / 0,29	0,21 / 0,22

[a] 1,2,3,4 – Look a technique
[b] Values are means of triplicates

Table 2. Microbiological characteristics of grey forest soil at application of organic and mineral fertilizers in 2002/2003

Variantsa	Bacteria x108 cell/g	AM fungi mkg/g	Fungi (other) mkg/g	Diversity indices of bacteria
May				
1	0,4 / 1,4	3,3 / 25,0	34,1 / 70,5	4,1 / 4,7
2	0,8 / 1,0	60,7 / 1,2	120 / 1,2	4,3 / 4,0
3	0,6 / 2,1	5,5 / 72,7	104 / 72,7	4,7 / 4,8
4	0,7 / 3,3	33,8 / 28,9	94,2 / 29,0	3,8 / 4,4
August				
1	1,0 / 1,2	<1 / 34,6	30,24 / 66,3	3,4 / 4,8
2	0,9 / 2,7	<1 / 118	<1 / 49,6	3,9 / 4,9
3	0,9 / 1,0	22,5 /56,7	14,9 / 54,6	3,5 / 4,8
4	1,6 / 0,8	21,3 /17,9	13,2 / 3,4	3,6 / 3,6

[a] 1,2,3,4 – Look a technique

Conclusion

Application of manure (40 t/ha) and manure with addition of nitrogen (30 and 60 N_{aa} kg/ha) promotes increase in a microbial diversity of microorganisms in grey forest soil in early period of vegetation of grain crops (winter wheat, winter rye and spring wheat). In the end of the vegetative period levelling influence of manure on a microbial diversity of investigated soil which became similar with a complex of microorganisms in a variant with application of full mineral fertilizer into doses N120P60K60 and N140P50K190 was observed.

Cultivation of an oats on a background after-action of manure without application of mineral fertilizers and at application of full mineral fertilizers (N30P50-60K90-120) promotes increase in a microbial diversity of grey forest soil and a total number of microorganisms.

Cultivation of a potato on a background of application of mineral fertilizer in dose N140P50K190 is accompanied by decrease in a total number of microorganisms (in 4 times) and a bacterial diversity by the end of vegetation. Positive correlation of Shannon diversity indices with the contents in the soil of AM fungi testifies to stimulating influence of the latest on a biodiversity of microorganisms of grey forest soil.

The insufficient humidification (low hydrothermal factor) creates conditions for primary development of aerobic actinomycetes (*Micromonospora* sp., *Rhodococcus equi*, *Nocardia asteroids*, *N. carnea*) and coryneform bacteria (*Arthrobacter globiformis*). Increased humidifying of the soil promote to development of anaerobiosis - in community of microorganisms the content of anaerobic bacteria with fermenting type of a metabolism (*Aeromonas hydrophila, Clostridium pasterianum*, *Propionibacterium freudenreichii*, and also of the some anaerobic coryneform bacteria (*Butyrivibrio spp.*) has increased.

References

Hu S.J., Van Bruggen A.H.C., Crünwald N.J. Dynamics of bacterial populations in relation to carbon availability in a residue-amended soil//Appl. Soil Ecol. – 1999. – V. 13, pp. 21-30.

Pascual J.A., Garcia C., Hernandez T., Moreno J.L., Ros M. Soil microbial activity as a biomarker of degradation and remediation processes//Soil Biol. Biochem. – 2000. – V.32, pp. 1877-1883

Peacock A.D., Mullen M.D., Ringelberg D.B., Tyler D.D., Hedrick D.B., Gale P.M., White D.C. Soil microbial community responses to dairy manure or ammonium nitrate applications//Soil Biol. Biochem. – 2001. – V.33, pp. 1011-1019.

Ajwa H.A., Tabatabai M.A. Decomposition of different organic materials in soils //Biol. and Fertil. of Soils. –1994. – V.18, pp.175-182..

Giller K.E., Beare M.N., Lavelle P., Izac A.-M.N., Swift M.J. Agricaltural intensification, soil biodiversity and agroecosystem function//Appl. Soil Ecol. – 1997. – V. 6, pp.3-16.

Kandeler E.,Luxhøi J., Tscherko D., Magid J. Xylanase, invertase and protease in the soil-litter interface of a loamy sand//Soil Biol. Biochem. – 1999. –V.31, pp.1117-1179

Graham J.H., Hodge N.C., and Morton J.B. Fatty Acid Methyl Ester Profiles for Characterization of Glomalean Fungi and Their Endomycorrhizae//Appl. Environ. Microbiol. – 1995. – V 61, pp. 58-64.

Stephen P. Bentivenga and Joseph B. Morton. Congruence of fatty acid methyl ester profiles and morphological characters of arbuscular mycorrhyzal fungi in Gigasporaceae//Proc. Natl. Acad. Sci. – 1996. USA. - V. 93, pp. 5659-5662.

Olsson P.A., van Aarle I.M., Gavito M.E., Bengtson P. and Bengtsson G. ^{13}C Incorporation into Signature Fatty Acids as an Assay for Carbon Allocation in Arbuscular Mycorrhiza//Appl. Environ. Microbiol. – 2005. – V.71, pp. 2592-2599.

Practical work on agrochemist /Edited by Mineev V. G. – M.: Publ. MGU. – 2001. – 689 pp.

Osipov GA, Turova ES. Studying species composition of microbial communities with the use of gas chromatography-mass spectrometry. Microbial community of kaolin. FEMS//Microb. Rev. – 1997. V.20, pp. 437-446.

The directory of the agriculturist on agricultural meteorologies/Edited by I.G.Gringof. – Gidrometeoizdat. - 1986. – 518 pp. 13.Kudeyarova A.Yu. Phosphatogenic transformation of soils. – M.: Nauka. - 1995. – 288 p.