ISSN 1068-3674, Russian Agricultural Sciences, 2007, Vol. 33, No. 2, pp. 100–102. © Allerton Press, Inc., 2007.
Original Russian Text © N.V. Verkhovtseva, E.N. Kubarev, V.G. Mineev, 2007, published in Doklady Rossiiskoi Akademii Sel'skokhozyaistvennykh Nauk, 2007, No. 2, pp. 26–27.

AGROCHEMISTRY. PEDOLOGY

Agrochemical Agents in Maintaining the Structure of the Soil Microbial Community

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Received November 20, 2006

Abstract—It is shown that aerobic–anaerobic equilibrium in the structure of the microbial community of soils promotes preservation of organic carbon when organic fertilizers (vermicompost) are applied. Changes in the structure of the microbial community under legume crops occur as a result of an increase in numbers of aerobic and anaerobic species of the nitrogen cycle (free-living and associative) and carbon cycle.

DOI: 10.3103/S1068367407020103

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 a rule, also increase [1–4]. The use of only organic fertilizers, however, can lead to rapid mineralization [5] of both applied (allochthonous) and own (autochthonous) organic matter as a result of disturbing metabiosis—the interrelation of food chains of processing nutrient substrates by individual organisms of the agroecosystems, and the use of only mineral fertilizers can cause a decrease of the biomass of microorganisms in soil [6] even if there is no acidification of the soil solution. The combined used of these fertilizers provides a positive balance of organic matter, 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 [7]. Consequently, data not only on total numbers of microorganisms but also on the structure of the microbial community of soils, notions about which are broadening in connection with the use of molecular methods of investigating their microbocenoses, are important.

The purpose of the work was to assess the effect of organic (vermicompost VC obtained by processing semi-rotted cow manure by the manure worm *Eisenia foetida*) and mineral fertilizers on a change in the microbial community of alluvial gray humic gleyish soil and content of organic matter and nitrogen in it during successive growing of potatoes and a vetch–oat mixture.

METHODS

The investigations were conducted in 2004–2005 in the territory of the Kovrovskii Agricultural Production Cooperative (Vladimir oblast). The design of the field experiment included variants: (1) control, (2) VC in a dose of 15 t/ha, (3) VC + $N_{90}P_{90}K_{90}$ (VC + NPK), and (4) $N_{90}P_{90}K_{90}$ (NPK). Replication of the experiment was fourfold. The area of the experimental plot was 28 m^2. To determine the agricultural characteristics of the soil, we selected a mixed sample from the plow layer (0-25 cm). Fertilizers were applied before plowing in the spring by surface broadcasting prior to planting potato variety Udacha.

The soil content of organic carbon (C_{org}) and total nitrogen (N_{tot}) was measured [8]. The numbers of various groups of microorganisms determined by the molecular gas chromatography–mass spectrometry (GC–MS) method, making it possible after acid methanolysis of the soil sample to isolate specific biomarkers of cells of microorganisms—fatty and hydroxy acids and aldehydes—from complex lipids of cell membranes in which they are bound by ester and amide bonds. Then, using the calculation program developed earlier [9] and the database on these biomarkers, we determined the biomass and diversity of the microbial communities. The analytical data were processed by the computer statistical program Microsoft Excel 2000.

RESULTS AND DISCUSSION

The soil on which the field experiment was conducted was characterized by a rather high C_{org} content (1.62%) and narrow C : N ratio, 7.4. Losses of C_{org} were not observed after harvesting the potatoes, and the content of organic matter in the soil increased considerably (by 26%) compared with the control after growing the vetch–oat mixture.

A detailed examination of the structure of the soil microbial community made it possible to determine the species of microorganisms promoting preservation of autochthonous organic matter and C : N, the balance. Thus, microbial diversity in the soil after harvesting potatoes is represented by 44 species of microorganisms with rather high numbers (table). The microbial assemblage consists of 50–60% anaerobic and facultative anaerobic bacteria, both in a species respect (26 of

Numbers of microorganisms after harvesting potatoes (1) and vetch–oat mixture (2)

Microorganisms, cells/g × 10^6	Control		VC		VC + NPK		NPK	
	1	2	1	2	1	2	1	2
Acetobacter diazotrophicus	17.6	92.5	14.9	112.0	14.9	85.8	12.6	121.1
Agrobaterium radiobacter	1.4	0	0.9	0	0.7	0	0.8	0
Methylococcus sp.	5.2	14.0	3.7	14.6	4.6	6.4	2.9	18.3
Pseudomonas fluorescens	3.3	9.4	1.9	7.9	2.4	4.1	2.3	6.8
P. putida	2.5	11.0	1.7	8.9	2.2	5.1	2.0	8.8
P. vesicularis	1.6	11.7	1.4	12.4	1.5	8.3	1.3	10.7
Sphingobacterium spiritovorum	0.9	3.0	0.6	3.3	0.7	0.4	0.6	3.3
Sphingomonas capsulata	1.2	4.0	0.8	4.0	0.9	2.8	0.8	4.1
Xanthomonas sp.	2.3	13.5	1.9	13.9	2.0	9.3	1.8	13.4
FeRed	0.2	2.3	0.1	3.6	0.2	3.5	0.1	3.0
Aeromonas hydrophila	3.7	45.1	3.1	45.2	5.0	34.2	3.5	44.7
Bacteroides fragilis	1.2	6.8	2.5	9.7	1.0	7.6	0.9	10.6
Bacteroides hypermegas	0.2	0.8	0.1	0.9	0.1	0.6	0.1	0.8
Bacteroides ruminicola	2.1	8.4	1.5	10.3	1.6	6.6	1.6	9.3
Wolinella sp.	3.8	23.3	4.3	26.9	3.3	23.1	2.8	28.2
Desulfovibro sp.	1.5	0.3	0.4	0	0.1	0	1.3	2.9
Chlamydia sp.	0.1	0.3	0.1	0.5	0.1	0.6	0.1	0.8
Nitrobacter sp.	0.3	0	0	0	0.1	0	0.3	0
Cytophaga sp.	2.3	10.1	0.8	11.5	1.9	8.6	1.6	11.1
Arthobacter sp.	1.5	0.7	0.6	0	0.6	0	0.4	0.9
Starhylococcus sp.	2.9	4.7	2.0	6.5	2.1	4.2	2.8	2.9
Bacillus sybtilis	2.7	6.2	1.4	4.7	1.9	2.7	1.6	8.1
Bacillus sp.	4.5	3.6	3.1	5.4	3.1	0	3.4	6.3
Clostridium dificile	0	0	0	1.3	0	2.2	0	0
C. pasteurianum	5.2	14.0	3.7	14.6	4.6	6.4	2.9	18.3
C. perfringens	0.4	0.8	0.2	1.3	0.3	0.6	0.3	1.1
C. propionicum	1.3	2.5	1.5	2.3	1.5	0.6	1.2	2.8
Acetobacterium sp.	0	1.4	0.1	3.8	0.5	1.1	0.1	0
Butyrivibro 1-2-13	3.1	11.3	2.3	13.2	0	6.0	2.5	12.7
Butyrivibro 1-4-11	8.4	7.5	5.7	10.1	6.7	0.3	6.9	12.8
Butyrivibro 7S-14-3	20.5	26.3	16.7	36.2	17.6	1.8	16.4	51.2
Bifidibacterium sp.	4.2	0	3.4	0	3.0	0	4.2	2.3
Corynebacterium sp.	0	0.4	0	0	0	2.7	0	0
Eubacterium lentum	2.4	2.9	1.7	4.0	1.8	0.3	1.9	4.6
Eubacterium sp.	0.2	0.1	0.1	0.2	0.1	0.2	0.1	0.3
Propionibacterium freudenreichii	1.5	0	0.7	0	0.1	0	0.6	0
Propionibacterium sp.	18.5	1.8	1.9	5.0	3.7	4.7	4.0	13.7
Rhodococcus sp.	10.4	32.4	6.1	25.0	6.8	11.9	6.7	17.1
R. terrae	3.5	0.6	2.3	1.3	2.2	0.4	2.5	1.9
Ruminococcus sp.	18.1	34.1	12.8	64.2	15.4	3.5	12.4	51.6
Pseudonocardia sp.	1.4	2.4	1.0	2.7	0.9	1.4	1.0	3.6
Streptomyces sp.	0	4.5	0	3.5	0	6.0	0	0
Nocardia carnea	0.8	0.6	0.5	0.9	0.8	1.0	0.5	0.7
Actinomadura roseola	0.8	0.5	0.3	1.1	0.3	1.2	0.5	1.8

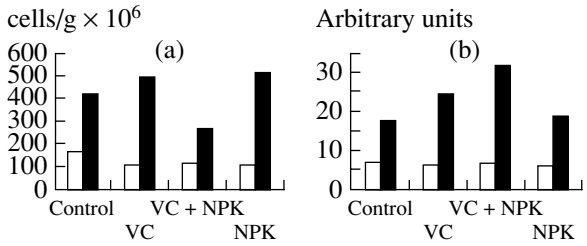

Fig. 1. Numbers of bacteria (a) and fungi (b) in soil according to GC–MS data after harvesting potatoes (light column) and vetch–oat mixture (dark column).

44 detected species) and with respect to biomass. With the use of mineral fertilizers, their total numbers decreased mainly due to certain anaerobic species, in particular, propionibacteria (*Propionibacterium freudenreichii*), bacterioids, clostridia, and iron bacteria. The numbers and members of actinobacteria (genera *Rhodococcus*, *Pseudonocardia*, and *Actinomadura*) decreased insignificantly. The decrease in numbers of some of these representatives can be considered positive. Thus, *P. freudenreichii* and iron bacteria can use humic compounds as a carbon and energy source, in which case autochthonous C_{org} is lost [10]. Bacterioids and clostridia can accumulate is soil volatile fatty acids, particularly butyric, which is unfavorable for homeostasis of the agrocenose. Consequently, mineral fertilizers perform a protective function, since they prevent the development of the given species of anaerobes.

After harvesting the vetch–oat mixture, total numbers of soil bacteria and biodiversity in all variants increased by 2.5–4.7 times (figure) due to aerobic and anaerobic species (table). Among aerobes, the group of pseudomonads (*Acetobacter diazotrophicus, Pseudomonas fluorescens, P. putida*, etc.) increased substantially (by 3–5 times), many of which are associative nitrogen fixers and their multiplication is stimulated by plants, in particular, by legumes. Of the anaerobic species, the numbers of *Clostridium pasteurianum*, a free-living nitrogen fixer, and *Aeromonas hydrophila* and *Wolinella* sp., associative nitrogen fixers, increased significantly. The content of aerobic (*Cytophaga* sp.) and anaerobic (*Bacteroides* spp.) species able to decompose cellulose increased noticeably. We can conclude a considerable role of plants of the vetch–oat mixture in stimulating the multiplication of microorganisms, since in the control variant (without fertilizers) a more than twofold increase in numbers of microorganisms occurred.

The high biofertilization properties of exudates of legumes and their residues, promoting a substantial increase of biodiversity and numbers of soil microorganisms, have been pointed out in many publications [11]. Evidently, such an enrichment of the soil with various organic substrates of plant origin, which increase its microbial status, is observed in our experiment. Maximum numbers of bacteria after harvesting the vetch–oat mixture are noted in the variant with complete mineral fertilizer (Fig. 1a), apparently as a consequence of a better balance of processes of mineralization, synthesis, and resynthesis of C_{org} of plant and microbial origin. The mineral background is also favorable for normalizing the content of microscopic fungi (Fig. 1b), which is commensurate with their numbers in the control variants and almost half as many than with the use of VC against an NPK background.

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