"EFFECTS OF DIFFERENT ORGANIC FERTILIZERS AND DOSES ON SOIL MICROFLORA DYNAMICS"

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Abstract
The paper presents the effects of two different organic fertilizers, "Aibios" and "Biorga", and green manuring, clover (*Trifolium alexandrinum* L) and horse bean (*Vicia faba* L) + ryegrass (*Lolium multiflorum* Lam), on soil microflora dynamics, bacteria, actynomycetes and fungi, responsible for soil microbiological activity such as mineralization of organic matter into assimilated elements for plants, atmospheric nitrogen fixation, phosphorus mobilization, etc. The study was carried out during three consecutive years, 2008-2010, in a vineyard in Romanat, Durrës, where were tested seven variants of different fertilizers and doses: V_1 = control - no fertilizers were used, V_2 = "Aibios" 10 kv/ha, V_3 = "Aibios" 15 kv/ha, V_4 = "Biorga" 15 kv/ha, V_5 = "Biorga" 20 kv/ha, V_6 = clover 200 kv/ha fresh mass, and V_7 = horse bean + ryegrass 200 kv/ha fresh mass. Microbiological soil tests showed that the application of different organic fertilizers and doses has significantly affected the soil microflora dynamics and its activity. It was found a significant raise of the total microflora up to 132.1% (V_7), compare to control. The aerobic bacteria populations constitute 87.8-91.3% of the total microflora. Application of organic fertilization has significantly affected the amount of released CO_2 and nitrogen fixation ability. The highest levels of released CO_2 were measured in variants V_6 and V_7 by 13.2 and 14.8 mg/100 g soil/96 hours, respectively. The highest levels of the quantity of nitrogen fixation were measured for V_6, V_7 by 13.4 and 11.9 mg/100 g soil/24 hours or 106.1% and 83.1%, higher than control. The amount of released CO_2 and fixed nitrogen can be used by plants following a higher grape production.

Key words: *Aibios, Biorga, clover, green, microflora dynamics, organic manure.*

Introduction

Intensive cultivation, which leads to depletion of soil nutrients, is a major constraint on food production. To overcome the rapid decline in soil fertility, inputs of organic materials such as manure, compost and animal dung that contain nutrients are applied to the soil to improve and maintain crop yield. VanCotthem (2007) defines fertilizer as any organic or inorganic material of natural or synthetic origin which is added to a soil to augment the level of or supply certain essential element for plant growth. Any amendment to the soil to enhance soil fertility is therefore a fertilizer. An organic fertilizer is derived from materials that are essentially carbon source in nature. These materials of organic fertilizers can either be plant or animal or their by-products. One distinct merit of organic fertilizers is that they naturally contain organic matter that is beneficial to plants and the soil. Organic matter in organic fertilizers helps to improve the water-holding capacity of soil and also augments its structure, thus increasing its nutrient holding capacity as well. Another benefit of organic matter in organic fertilizers is that it encourages microbial activity which plays a crucial part in the breakdown of nutrients so that plants can use them (Nester *et al*., 1998). There is less danger of over-fertilization by adding decomposed organic material to a garden. According to Paul and Clark (1996), it provides a slow release of nutrients as micro-organisms in the soil break down the organic material into an inorganic and water soluble form which the plants can use. The ultimate goal of fertilizer application is to maximize productivity and economic returns.

Several studies have shown that application of organic fertilizers reduces the incidence of soil-borne diseases and pathogens (Faqir *et al*., 1995; VanLauwe *et al*., 1996; ACMSF, 1998; Sarathchandra *et al*., 2001; Graham and Haynes, 2005).
Mobambo et al. (1994) conducted surveys of field and compound plantain plantings in Nigeria to determine effects of application of organic fertilizer on the severity of black leaf streak (*Black sigatoka*) disease caused by *Mycosphaerella fijiensis*. They noted that the disease was reduced in compound gardens where organic fertilizers were regularly applied in the form of household refuse and animal waste. VanLauwe et al. (1996) observed an increase in soil biomass when readily decomposable organic matter was added to native soil. The effect of long-term application of organic fertilizers on soil microbial populations can be measured either as changes in the population of a particular organism, organism groups or methodologically defined pools such as the microbial biomass or as changes in biological activity, for example, soil respiration and enzyme activities. Variable effects of a given amendment on different organisms may change the composition of the microbial community without changing total populations or activities (VanZwieten, 2006).
Researches on practices of soil fertilization systems have shown that the quality of soil is determined by the use of organic fertilizers and plant residues sufficient to create a stable environment for biological activity and a microbial ecology different from the soil under conventional practices (Al-Ghazali *et al*., 1990). Thus, an increased ratio of microbial diversity in the end turns into a sustainable use of humus, thus creating a higher degree of aggregation with a positive impact on air regime, water and soil thermal ductile horizon. The first in this aspect, the system of fertilization according to the principles of biological agriculture, creates a sustainable agriculture and safe environmental conditions.
Use of organic fertilizers is associated with the prevalence of species of microorganisms that are responsible for the mineralization of organic substances; mobilize phosphorus from insoluble mineral forms of organic material and atmospheric nitrogen fixation (Badarudolin & Moyer, 1990).
Based on the foregoing, the use of organic fertilizers "Aibios" and "Biorga", processed by biological methods, and green manure during the winter dormancy of plants is seen as a means of the increase of soil fertility potential, getting the maximum production of high quality and the creation of a clean and sustainable terrestrial environment.
The purpose of this study was to assess the population dynamics of resident soil bacteria and fungi in relation to the period of organic fertilizer application.

Material and methods

The experiment was conducted in three consecutive years, 2008- 2010, in a 12 years old vineyard, planted with an autochthonous grape cultivar “Shesh i Zi”, in Romanat, Durrës, in the central part of Albania. The plot was situated in a uniform hill with a sloping gradient of 5 to 6%, and a planting density of 3330 plants ha^{-1} (2.5 m x 1.2 m). A randomized complete block design (RCBD) with 7 variants and 4 replications and plot size of 50 m^2 containing 17 vines was used. The following variants were studied:
V_1 – control (no any fertilizer was used)
V_2 - organic manure "Aibios", 10 kv ha^{-1}
V_3- organic manure "Aibios", 15 kv ha^{-1}
V_4 - organic manure "Biorga", 15 kv ha^{-1}
V_5 - organic manure "Biorga", 20 kv ha^{-1}
V_6 - green manuring with clover 200 kv ha^{-1}, fresh mass.
V_7 - green manuring with horse bean + ryegrass, 250 kv ha^{-1} fresh mass.
Organic fertilizers, "Aibios" and "Biorga" were applied during winter dormancy of the

grapevine, on the period February 20 to March 15, and green manure, clover and horse bean + ryegrass, were applied on the period June 10-15. Seven soils samples (one representative sample for each variant), were tested every experimental year for soil chemical and microbiological indicators, approximately in the middle of the grapevine vegetative period, which corresponds on 20^{th}-30^{th} July. Laboratory tests were conducted at the Analytical and Microbiological Lab of the Agricultural Technology Transfer Center of Fushë Kruja.
For microbiological indicators there were applied dilutions 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8}, a procedure that was performed to facilitate counting and identification of dominant species. Petri dishes and test tubes planted with sample volumes, according to specific dilutions, were incubated in the thermostat at temperature 28-30^{o}C for 3-15 days, depending on the systematic and physiological groups of microorganisms. The final counting of full-grown colonies was conducted at the end of the incubation of each microbial population group. There were used elective and selective medium for each group of microorganisms. Species identification was based on macroscopic characteristics of colonies and observation under the microscope (Immersion with 1000X magnification), using the respective catalogs.

Results and discussions

Microbiological analysis data show a significant increase in biological and enzymatic activity in the all treated variants with organic fertilizers, "Aibios", "Biorga" and green manuring, compare to control (untreated variant). There was found a significant increase of the total microflora in the variants 3, 6 and 7, with an increase of 42.7%, 89.7%, and 132.1%, respectively. As Albiach, et al. (2000) noticed, in the case of organic manure application, the raise of total soil microflora comes as a result of the organic mineralized carbon, nitrogenous compounds and aromatic alchyl compositions, while, in the case of green manuring it comes from high content of easily biodegradable nitrogen compounds, lignine and cellulose in plant biomass of clover, horse bean and lolium. In variants 2 and 4, where were used minimal doses of organic fertilizers "Aibios" and "Biorga", there was observed only a tendency of the increase of biological and enzymatic activity of the soil.
Aerobic bacteria population constitutes the largest number of the total microflora (or 87.8-91.3%), from 13.1 million to 32.1 million cells/g dry soil, with a significant difference between the variants. The population of aerobic bacteria in variants 3, 6 and 7 was 41.3%, 94% and 141.4%, respectively, higher than control.
Organic and green manuring increased species diversity of aerobic bacteria, indicating a dominance of bacteria of the genera *Pseudomonas sp.*, *Pseudomonas floreches* and *Bacterium sp.*, which contribute to a higher pH of the rhizosphera area and a higher mobilization of phosphorus (P_2O_5) from insoluble phosphorus forms such as $Ca_3(PO4)_2$ in a range of 23-29% (Wander, 1994). The highest number of microorganisms that use organic nitrogen compared to those which use mineral nitrogen is an indicator of the high degree of the organic matter mineralization and enzymatic activity as a result of vital activity of soil microorganisms. This also indicates a greater amount of N-NO_3 and N-NH_4 accumulation.
There were observed significant differences between the variants concerning to actinomycetes (anaerobic bacteria). The number of actinomycetes range from 1.8 million to 3 million cells/g dry soil and highest values were found on variants 3, 6 and 7. Actinomycetes play an important role in the formation of humus and fulvoacids, because of their vital activity. According to various researchers (Tauxe *et al*., 1997; Olivian & Steinberg, 2006), the synthesis of humus substances is being catalyzed by polyphenol-oxidase which is synthesized by actinomycetes, and, in order to have a positive balance of the humus in the soil, must apply rotary and fertilization system on the farm to respond to the quantity of the

mobilized (dissolved) humus. Changes of the soil in microbiological terms are dictated by the provided organic matter in quantity and rich in composition, humus substances, growth stimulants and antibiotics that are of biological origin of the all organic fertilizers that have helped in providing a better hydro, thermal and aerial regime.

Fungi were found at moderate levels in all variants. In the case of the use of organic fertilizers such as "Aibios" and "Biorga" dominated *Trichoderma sp*. (*Trichoderma harzianum*, *Trichoderma hamatum*), while, in the case of the green manure, clover and horsebean + lolium, dominant species were *Penicillum sp*., *Aspergillus sp*., *Trichoderma viridi*, *Mucor sp*., *Dematinum*, *FOMA* and *Stachybotris* (Ballesteros - Sandoval, 1999). Diversity of the identified species in soil samples representing variants 2, 5, 6 and 7 assume an increase of bio-control to plant diseases and pests and phytosanitary potential of the soil. Increased loads of *Aspergillus sp.* and *Penicillium sp.* assumed an increased level of mycotoxins in the environment with impact on human and animal health (Strauch, 1991, FDA, 1998).

The numbers of sporal bacteria forms varies from 510 to 602 thousand cells/g dry soil, and were found in increased levels in variants 3, 6 and 7, compare to control, while in variants 2, 4 and 5 was observed only an increase trend. Referred to the morphological data of developed colonies in Petri dishes, there was found that the most widespread sporal bacteria were *Bacillus megaterrium*, *Bacillus subtilus*, *Bacillus cereus*, *Bacillus mycoides* and *Bacillus mesentericus*. Variants treated with organic fertilizers and green manuring provided higher amonification potential. High levels of *Bacillus megaterrium* indicates a greater mobilization of phosphorus, while the presence of *Bacillus cereus* indicates an increased bio-control potential of plant diseases and pests (Tauxe *et al*., 1997; Olivian & Steinberg, 2006).

In the nitrogen cycle take part to many heteretrophic independent bacteria, which are able to convert gaseous atmospheric nitrogen (N_2) in forms that can be used by plants. Bacteria that provide the greatest amount of N_2 in the soil are *Azospirillum lipoferum*, *Azospirillum brazilence*, *Azotobacter croccocum*, *Azotobacter vinelandi*, etc. Microbial loads of free nitrogen fixation of variants 3, 6 and 7 were 82-103% higher than control, which means that these variants have the highest nitrogen fixation potential. Bacteria of *Azotobacter* genera synthesize polysaccharides that bind soil particles, helping to improve soil structure and better oxygen diffusion in the soil (Table 1).

Table 1. Systematic groups of microorganisms and microbial biomass, according to variants (mean values, different letters indicate significant difference at P<0.05).

Variants	Present microflora (000/g dry soil)				Estimated microbial biomass (kg/ha)	Soil humidity (%)
	Total microflora	Aerobic bacteria	Actinomycetes (anaerobic bacteria)	Fungi		
V_1	15.141 c	13.300	1.821	20	3.078 d	16.2
V_2	15.334 c	13.390	1.921	23	3.411 c	17.7
V_3	21.609 b	18.800	2.782	27	4.286 b	18.4
V_4	14.959 d	13.150	1.787	22	3.262 cd	16.5
V_5	17.773 bc	15.750	2.002	21	3.330 c	16.2
V_6	28.727 ab	25.798	2.900	29	4.801 ab	20.2
V_7	35.146 a	32.105	3.010	31	5.284 a	22.4

In variants where was used "Aibios" and green manuring, amonification activity was 2.2-2.5 times higher than control. These data indicate an accumulation of nitrogen in ammonium form, which is chatched to organic matter and clay particles and becomes more stable in soil than NO_3^- and NO_2^- nitrogen forms.

Nitrosomonas and *Nitrobacter* genera were raised in all fertilized variants (organic and green manuring), compare to control. The number of nitrification bacteria ranges from 15.2 to 36.7

thousand cells/g dry soil, for control and V_7, respectively. Nitrification bacteria loads in variants 3, 6 and 7 were 34.2%, 100.7% and 141.4% higher than control. These data indicate that ammonium nitrogen was converted almost entirely into NO_3^- and NO_2^- nitrogen.

In variants with organic and green manuring was observed also an increased denitrification activity. Microbial loads of denitrification bacteria in variants 6 and 7 were 25% and 28% higher than control, which means that these variants release 25% and 28% higher amount of nitrogen to the atmosphere, but since the N-fixation bacteria loads in these variants are higher (Table 2), the negative effect is not significant. In this point of view, biological denitrification is a detrimental process because the fact that it contributes to the impoverishment of the soil with nitrogen and in the global warming. According to different authors, because of microbial denitrification, there occur 24-44% losses of the biological soil accumulated nitrogen in forms of NO, N_2O and N_2 (Saggar *et al.*, 2000; VanLauwe *et al.*, 1996; Rangaraj *et al.*, 2007). Denitrification, as a process, is a more common phenomenon of biological and chemical degradation for which we must intervene to make it less harmful to agriculture, environment and humans (Freney & Simpson, 1983).

Use of organic fertilizers "Aibios" and "Biorga" and green manuring with clover and horsebean + lolium increased the cellulotic activity in the soil, as well. The number of microorganisms that participate in the decomposition of cellulose of variants 3, 6 and 7 was significantly increased compared to control. This result is similar to Campbell *et al.* (1993), Litterick, (2004), etc. At the same time, there was observed a significant increase of the microbial loads of the phosphorus bacteria, from 31.9 thousand (control) to 74.4 thousand cells/g dry soil, and the amount of assimilable phosphorus (P_2O_5) in the soil, compare to control (Table 2).

Table 2. Physiological groups of microorganisms, according to variants (mean values, different letters indicate significant difference at P<0.05).

Variants	Bacilli (000/g dry soil)	Bacteria (000/g dry soil)					
		N-fixation bacteria	Phosphorus bacteria	Amonification bacteria	Nitrification bacteria	Denitrification bacteria	Cellulose knockdowns
V_1	143	2.9 c	31.9 d	4100 d	15.2 d	800 c	20.9 d
V_2	197	3.2 c	46.1 c	6100 cd	19.1 cd	900 bc	25.1 cd
V_3	510	5.0 b	59.7 b	8990 b	20.4 c	910 bc	30.2 b
V_4	179	3.0 c	46.0 c	6300 c	19.0 cd	822 c	22.9 c
V_5	371	3.0 c	40.8 cd	6500 c	20.9 c	897 bc	23.8 c
V_6	574	5.9 a	71.0 ab	10120 a	30.5 b	1000 ab	34.1 ab
V_7	602	5.3 ab	74.4 a	9010 b	36.7 a	1024 a	37.3 a

Taking into consideration chemical indicators of the soil, there were observed significant differences between variants. In variants 3, 6 and 7, was found a little increase of pH, from 7.1 (control - neutral) to 7.75 (V_6 – lightly basic). There was a significant increase of organic mass (%) for variants 7, 6 and 3 of 2.03, 1.73 and 0.93%, respectively, compare to control. The highest levels of P_2O_5 (mg/100 g soil) and K_2O (mg/100 g soil) content were found for V_2 and V_3 maybe because “Aibios” contains high amount of P_2O_5 and K_2O. The highest levels of total nitrogen content (%) were observed for variants 5 and 3 of 0.158 and 0.156%, respectively. There were no significant diferences between variants 6 and 7 (green manuring) for almost all chemical indicators of the soil (Table 3).

Table 3. Chemical indicators of the soil, according to variants (mean values, different letters indicate significant difference at P<0.05).

Variants	Sample	pH	Humus	Organic	P_2O_5	K_2O	Total	$N0_3$ nitrogen

			(%)	mass (%)	(mg/100 g soil)	(mg/100 g soil)	Nitrogen (%)	(mg/100 g soil)
V_1	M1	7.1	1.28 d	7.43 d	0.79 d	13.8 e	0.084 d	22.4 d
V_2	M2	7.5	1.67 b	7.43 d	4.42 b	28.0 b	0.139 bc	28.0 b
V_3	M3	7.35	1.69 b	8.36 b	5.81 a	35.4 a	0.156 a	33.6 a
V_4	M4	7.3	1.47 c	7.70 c	3.02 c	17.2 d	0.136 c	23.6 bc
V_5	M5	7.1	1.58 c	7.98 bc	3.97 bc	19.1 d	0.158 a	24.4 bc
V_6	M6	7.75	1.98 a	9.16 a	3.95 bc	20.0 c	0.141 b	27.1 b
V_7	M7	7.7	2.01 a	9.46 a	4.36 b	22.7 c	0.142 b	27.3 b

CO_2 emission and nitrogen fixation ability

The amount of released CO_2 through *in vitro* cultivation methods (Jenkinson DS, 1988) showed significant differences between variants. It ranges from 6.9 mg/100 g soil (V_1 - control) to 14.8 mg/100 g soil (V_7) on 96 hours. The highest levels of released CO_2 were measured in variants treated with green manuring with clover (V_6), by 13.2 mg/100 g soil, and horse bean + ryegrass (V_7), by 14.8 mg/100 g soil, which assumes a greater mobilization of potassium and phosphorus, as well. This high CO_2 amount can be used by plants through photosynthesis following by a higher grape production. Use of organic fertilization (organic manure or green manure) was followed by a significant raise of nitrogen fixation. The highest levels of nitrogen fixation (mg/100 g soil/24 hours) were measured for V_6, V_7 and V_5, by 106.1% 83.1% and 69.2%, respectively, higher than control (Table 4).

Table 4. CO_2 emmission and nitrogen fixation (N_2), according to variants (mean values, different letters indicate significant difference at P<0.05).

Variants	Amount of CO_2 (mg/100 g soil of 96 hours)	N_2 fixation ability (mg/100 g/24 hours)
V_1 – Control	6.9 c	6.5 d
V_2 – Organic manure “Aibios” 10 kv/ha	9.9 b	8.9 c
V_3 – Organic manure “Aibios” 15 kv/ha	7.0 c	10.2 bc
V_4 – Organic manure ”Biorga” 15 kv/ha	6.9 c	9.1 c
V_5 – Organic manure ”Biorga” 20 kv/ha	7.6 bc	11.0 b
V_6 – Clover (green manuring)	13.2 ab	13.4 a
V_7 – Horse bean + ryegrass (green manuring)	14.8 a	11.9 ab

Conclusions

Application of different organic fertilizers and doses, as organic manure or green manuring, has significantly affected the soil microflora dynamics. There was found a significant raise of the total microflora in the variants 3, 6 and 7, with a raise of 42.7% to 132.1%, compare to control. The aerobic bacteria populations constitute the largest number of the total microflora (87.8-91.3%).

Application of different organic fertilizers was followed by a significant raise especially in some important species, such as bacteria, actynomycetes and fungi that mobilize nitrogen, phosphorus and potassium.

Application of organic fertilization has significantly affected the quantity of released CO_2 and nitrogen fixation ability. The highest levels of the amount of released CO_2 were observed on green manuring with clover and horse bean + ryegrass with 13.2 and 14.8 mg/100 g soil of 96 hours or 91.3 % to 114.5% higher than control, but since it can be used by grapevines through photosynthesis, there is no any significant negative impact to the atmosphere.

The highest levels of nitrogen fixation (mg/100 g soil/24 hours) were measured for V_6, V_7 and V_5, by 13.4, 11.9 and 11.0 mg/100 g soil/24 hours or 106.1%, 83.1% and 69.2%, higher than control. The amount of released CO_2 and fixed nitrogen can be used by plants following a higher grape production.

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