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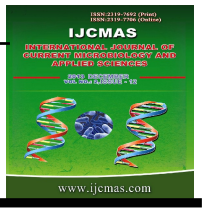

Original Research Article

The effect of solid cattle manure on soil microbial activity and on plate count microorganisms in organic and conventional farming systems

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A B S T R A C T

In Estonia, here is insufficiency of animal manure in organic arable farming. Green manure is used instead of farmyard manure amendments in stockless farms and the question is – is the green manure equal to farmyard manure, does it help to improve and sustain the soil biological properties and fertility. The aim of the research was to find out the influence of organic (ORGFYM – organic with green and cattle manure, ORGGRM – organic with green manure) and conventional (CONFYM – with green manure, cattle manure, mineral fertilizers and pesticides) cultivation systems on soil microbial activity, plate count microorganisms and on soil pH. Soil microbial biomass C (C_{mic}), N (N_{mic}) as well as soil basal respiration and metabolic quotient CO_2 (qCO_2) had no significant difference between the treatments. The dehydrogenase activity (DHA) was in ORGFYM treatment 16 – 20% higher than in other two treatments. Alkaline phosphomonesterase activity (APA) was highest in CONFYM and ORGFYM (treatments with manure) and 23 – 30% lower in ORGGRM (without manure). The results of trials showed that the application of cattle manure increased in the organic system (ORGFYM) the abundance of nitrification bacteria and the soil pH. Thus, although the green manuring is considered to be an important management practice in organic cultivation system to maintain and increase soil microbial activity and the abundance of microbes in different microbial populations, it is important to use also other organic fertilizers such as animal manure in addition to green manure.

Keywords

Organic farming; soil microbial biomass C (C_{mic}) and N (N_{mic}); soil basal respiration; dehydrogenase activity (DHA); alkaline phosphomonesterase activity (APA); plate count microorganisms.

Introduction

The diversity and abundance of life is in the soil more copious than in any other ecosystem. Microorganisms play a critical role in soil quality and support development of plants. They stimulate plant growth by facilitating the assimilation of phosphorus and iron, nitrogen fixation, releasing phytohormones, inhibiting root pathogens and synthesizing antibiotics (Glick, 1995).

Microbial communities adapt sensitively to changing environmental conditions by varying individual activity (Novak *et al*., 1993). The season, soil humidity, pH, fertilization and other factors predetermine the number and species composition of microorganisms in soil. For example, the supplement of organic fertilizers particularly stimulates bacteria and actinomycetes, reducing the fungal population (Novak *et al*., 1993). In some instances, changes in microbial communities can precede detectable changes in soil properties or in plant and animal communities, thereby providing an early sign of soil improvement or an early warning of soil deterioration (Pankhurst *et al*., 1995).

Numerous studies have indicated that organic farming has higher potential to accommodate biological concerns than conventional farming (Stolze *et al*., 2000). Plant production in organic farming mainly depends on nutrient release as a function of mineralization processes in soils. Therefore an active soil microflora and a considerable pool of accessible nutrients have priority in organic farming. So fertilizing the soil rather than the plant is an organic farmer's goal to assure sufficient nutrient mineralization in order to meet his economic needs (Fliessbach *et al*., 2000). The most important driving factors for these services are the amount and quality of organic manure and mulch, soil tillage, crop rotation, and crop diversity. Leguminoses in rotation supply symbiotically-fixed nitrogen to the system, aid in maintaining proper water status and reduce pathogen load (Perucci *et al*., 1997).

The development of Estonian organic farming began over 20 years ago in 1989. By 2010 organic land (121 815 ha) was about 13% of all agricultural land in use, but only about two thirds of organic farmers in Estonia keep animals (Vetemaa and Mikk, 2011). The greatest challenge for stockless organic farming is management of the nutrient supply. There is greater emphasis on alternative fertility building strategies, such as the use of green manure, and the import of manure, compost and other acceptable fertilizers. In Estonia, here is insufficiency of animal manure in organic arable farming. Green manure is used instead of farmyard manure amendments in stockless farms and the question is - is the green manure equal to farmyard manure, does it help to improve and sustain the soil biological properties and fertility?

The aim of this study was to evaluate microbiological activity and composition in soils in dependence on cultivation method. The cultivation methods carried out in the trial plots were: 1) organic (ORGGRM) with green manure like in stockless farming system 2) organic (ORGFYM) with solid cattle manure and green manure; and 3) conventional (CONFYM) – with green manure, cattle manure, mineral fertilizers and pesticides.

Materials and Methods

Experimental site.

The field trial was performed in Central-Estonia at Olustvere (58º 33´ N, 25º 34´ E). The soil type was *Podzoluvisol* (*PD*) according to FAO (1998). In the trial area the field crops have been cultivated according to the principles of organic farming since 2002. The conventional tillage was used in all treatment variants.

Experimental set up.

Since 2007 there was a five-field crop

rotation; winter rye (*Secale cereale* L., 2007), potato (*Solanum tuberosum* L., 2008), oats (*Avena sativa* L., 2009), barley (*Hordeum vulgare* L.) with undersown red clover (*Trifolium pratense* L., 2010), red clover (2011). The size of field was 1.2 ha, which was divided into three equal parts (4000 m^2) between the cultivation methods. Since 2007 the following cultivation methods were carried out: organic (ORGGRM) with green manure; organic (ORGFYM) with solid cattle manure and green manure; and conventional (CONFYM) – green manure, cattle manure, mineral fertilizers and pesticides were used (Table 1). Solid cattle manure (N 4.7, P 1.1, K 2.7 kg t^{-1}) at the rate of 60 t ha^{-1} was applied in fall 2007. The tillage method in all treatments was mouldboard ploughing to a depth of 20 cm in fall. Weeds were controlled after barley sowing by spring-tine harrowing.

Weather condition

A weather station situated in Viljandi (10 km from the study area) recorded data on air temperature and precipitation (Figure.1).

Soil sampling

Soil samples were taken on May 4th and September 15th 2010 (standing crop: barley). Soil samples (1 kg) from each treatment in four replications were taken by a random method from the 0 – 20 cm soil layer (plough layer) with a 1 cm ⌀ auger. Samples were sieved (2 mm) and air dried. Soil samples were kept at 4°C until they were analyzed in laboratory.

Soil analyses

Soil pH (H_2O) was measured according to the standard ISO 10390:2005.

Soil microbial biomass C (C_{mic}) and N (N_{mic}) were estimated by chloroform fumigation extraction (CFE) in accordance with Vance *et al*. (1987). CFE was done in triplicate on 20 g (dry matter) sub-samples that were extracted with 80 ml of a 0.5 M K_2SO_4 solution. Total organic carbon (TOC) in soil extracts was determined by infrared spectrometry after combustion at 850°C (DIMA-TOC 100, Dimatec, 45276 Essen, DE). Total N was subsequently measured in the same sample by chemoluminescence (TNb, Dimatec). Soil microbial biomass was then calculated according to the formula:

$$C_{mic} = E_C / k_{EC},$$

where E_C is TOC in fumigated samples – TOC in control samples and k_{EC} = 0.45 (Joergensen and Mueller, 1996a), and

$$N_{mic} = E_N / k_{EN},$$

where E_N is N_t in fumigated samples – N_t in control samples and k_{EN} = 0.54 (Joergensen and Mueller, 1996b).

Soil respiration was measured in preincubated (7 days at 22°C) samples as CO_2 evolved over a period of 164 h. Soil samples (20 g dry matter) were weighed into perforated centrifuge tubes and placed into a screw bottle (Schott, 250 ml) in the presence of 0.025N NaOH as CO_2- trap for a 24 h preincubation period in the bottle. The actual measurement started by adding exactly 20 ml of 0.025N NaOH. Exactly after 164 h the soil was taken out from the bottle and the alkali was titrated with 0.025N HCl. The measurement was done according to the reference methods of the Swiss agricultural research centre (FAL *et al*., 1996). The metabolic quotient for CO_2 (qCO_2) was calculated from basal respiration rates divided by the amount of

microbial biomass carbon (C_{mic}) in the respective sample (Anderson and Domsch, 1993)

Dehydrogenase activity (DHA) was measured in accordance with Tabatabai (1982) in 5 g soil samples incubated at 30°C for 24 h in the presence of an alternative electron acceptor (triphenyltetrazoliumchloride). The red-tinted product (triphenylformazan) was extracted with acetone and measured in a spectrophotometer at 546 nm.

Alkaline phosphomonesterase activity (APA) was measured following p-nitrophenol release from P–nitrophenyl phosphate. This method was suggested by Tabatabai and Bremner (1969) and modified by Margesin (1993) for an incubation temperature of 37°C. The assays of the phosphatases only differ in the choice of the pH-value of the buffer (6.5 and 11).

All soil samples were examined microbiologically for total number of bacteria, molds, yeasts, mesophilic spore-forming bacteria, *Fusarium* spp., actinomycetes, azotobacteria, cellulose decomposers, denitrifying and nitrifying bacteria using the plate-count method. Decimal dilution series were prepared in accordance with EVS-EN ISO 6887-1:2001. Microbiological counts were expressed as a number of colony forming units (CFUs) g^{-1} of dry soil. Plate Count Agar was used for isolation of total number of bacteria at 30°C for 72 h (NMKL No 86, 3rd ed., 1999 and ICC No. 125, 1978). For yeasts and molds the wort-agar medium were used at 25 °C for 5-7 days (ICC Standard No.146, 1992.). The total number of aerobic mesophilic spore-forming bacteria was estimated on the spore medium at 30°C for 72 h (ICC No.144 1992). The number of Fusarium spp. was defined on Nash & Snyder culture medium (Booth, 1971; Gerlach and Nirenberg, 1982). To identify the azotobacteria the Ashby culture media were used. The cellulose decomposers were defined on Hutchinson culture medium and nitrifying bacteria on water agar**.** For denitrifying bacteria the Hiltay culture media was used (Viileberg, 1966).

Data analyses

All results were based on four or three (plate count microorganisms) soil replicates. The data were analyzed by ANOVA. The Tukey-Kramer Honestly Significant Difference (HSD) test was used, effect of treatment on soil microbial activity and treatment and sampling date and their interaction on plate count microorganisms and pH were tested, using the software JMP 5.0.1.2 (SAS, 2002 JMP; SAS Institute, Cary, N.C.)

Results and Discussion

Soil microbial biomass

Microbial biomass is among the most labile pools of organic matter and it serves as an important reservoir of plant nutrients, such as N and P (Marumoto *et al*., 1982). Microbial biomass, in response to environmental changes, can therefore have important implications for nutrient bioavailability. The same results were also obtained by Melero and his colleagues (Melero *et al*, 2006).

There were no significant differences between the treatments (Table.2). However, the results showed tendency of greater soil microbial biomass in ORGFYM (with cattle manure). Microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}) were slightly higher in organic ORGFYM treatment (Table 2).

The C_{mic}/N_{mic} ratio is often used to describe the structure and state of the microbial community. Paul and Clark (1996) indicated that bacteria had a C/N ratio as low as 3.5 and fungi had the values from 10 to 15. Gunapala and Scow (1998) were found that C_{mic}/N_{mic} ratio was higher in conventionally than in organically managed soils, suggesting that bacteria were more abundant than fungi in organically managed soil.

In our study, the C_{mic}/N_{mic} ratio values in organic treatments were similar (ORGFYM – 5.62; ORGGRM – 5.63) and slightly but not significantly lower (5.35) in CONFYM treatment (Table 2). The C_{mic}/N_{mic} ratio value 5.35 – 5.63 suggests that in all treatment soils the bacteria were more abundant than fungi.

Soil respiration and enzymatic activity

Soil respiration in all three treatments was similar, indicating similar soil microbial activity in organically and conventionally managed treatments (Table 2). The *q*CO2 provides a measure of the specific metabolic activity that varies according to the composition and physiological state of the microbial community, the availability of substrates, and various abiotic factors (Anderson, 1994). This quotient has been proposed as an indicator of ecosystem disturbance during the adaptation of a system to different agricultural practices (Anderson and Domsch, 1990). The *q*CO2 alike soil respiration did not showed difference between the treatments. In all three treatments it was remarkably high. Under unfavorable conditions, the organisms require more energy to sustain the biomass, therefore, *q*CO2 values enhanced and the carbon is lost. High *q*CO2 values indicate stress (Fliessbach *et al*, 1994). An increased *q*CO2 apparently indicates stress to the soil microbial community, as any disturbance to an ecosystem is shifting energy from growth to maintenance (Odum, 1985). Agnelli *et al*. (2001) attributed high soil *q*CO2 to less availability of soil nutrients, whereas Pascual *et al*. (1997) indicated that soil under organic practices had a small value of *q*CO2 due to the protective capacity of organic matter on microbial biomass. Hopkins and Shiel (1996) found that in conventional plots, a smaller microbial community respired at a greater rate.

The dehydrogenase activity (DHA) was in ORGFYM treatment 16 – 20% higher than in other two treatments (Table 2).

Alkaline phosphomonesterase activity (APA) was significantly higher in the treatments were the solid cattle manure was used (ORGFYM, CONFYM) compared to ORGGRM (Table 2).

Soil pH and plate count microorganisms

Soil pH is one of the most influential factors in soil, and strongly influences the biomass, activity and composition of the microbial community (Matthies *et al*., 1997; Blagodatskaya and Anderson, 1998; Lauber *et al*., 2008; Jones *et al*., 2009; Edesi *et al*., 2012).

The results showed slightly higher soil pH in ORGFYM treatment (pH 7.18, Table 3) and lower in CONFYM treatment (pH 6.68). Also Mäder and his colleagues found in their study (Mäder *et al*., 2002) slightly higher soil pH in organic systems. This supports the knowledment that use of the mineral nitrogen fertilisers tends to acidify soils.

In September in all treatments the pH was lower than in spring. One reason could be the higher precipitations (105.2 mm) in September. During high rainfall the water

passing through the soil leaches basic cations into drainage water. These basic cations are replaced by acidic cations such as aluminum (Al^{3+}) and hydrogen (H^{+}). For this reason, soils pH decreases under high rainfall conditions (Hallik, 1963).

Total number of bacteria

The abundance of total number of bacteria did not differed between treatments. However, it was 94% higher in fall compared to spring (Table 3). The results of the study showed tendency that the fertilization with manure in ORGFYM treatment had positive aftereffect on the total number of bacteria.

Molds

It is known that the molds derive energy not through photosynthesis but from the organic matter in which they live. Typically, molds secrete hydrolytic enzymes, mainly from the hyphal tips. These enzymes degrade complex biopolymers such as starch, cellulose and lignin into simpler substances which can be absorbed by the hyphae. In this way, molds play a major role in causing decomposition of organic material, enabling the recycling of nutrients throughout ecosystems (Madigan *et al*, 2003).

Although the abundance in different treatments was similar and statistically significant differences did not occurred there was tendency that in ORGFYM treatment the abundance of molds was 38 – 71% higher than in ORGGRM and CONFYM treatments.

In general, the abundance in all treatments was higher in fall and lower in spring. It is well known, that the warm and humid weather is favorable for the growth of molds.

Yeast

Yeasts are important members in many ecosystems and form a significant contribution to biodiversity (Fleet, 1998). The soil is the ultimate repository for storage and even development of certain species of yeasts (Phaff and Starmer, 1987). Most of the yeast species possess a wide spectrum of metabolic abilities, enabling them to utilize many of the hydrolytic products of plant materials generated by fungal and bacterial activities (Phaff and Starmer, 1987).

In previous study we found yeast were very sensitive for the use of pesticides (Edesi *et al*., 2012). Also the Dickinson (1973) reported that some of the fungicides reduced soil yeast population. In present study in CONFYM treatment only once the herbicide was used and no other pesticides were used at all. For some reason, there was even tendency for greater yeast abundance on this treatment (Table 3).

Mesophilic spore-forming bacteria.

Spore-forming bacteria are versatile microorganisms able to produce spores highly tolerant to adverse environmental conditions, *e.g*., high temperature and drought (Gorlach-Lira, Coutinho, 2007) and intensive fertilizer and pesticide application (Bigelow *et al*. 2002). Also, the results showed similar abundance in all treatments (Table 3).

Whereas their abundance depended on the sampling time - it was higher in fall like with total number of bacteria and molds.

Table.1 Pesticides and mineral fertilizers use in conventional (CONFYM) treatment 2007–2010

Year	Date	Crop	Chemical category	Commercial name	Active ingredient/plant nutrient	Hectare amount
2007	9. April	winter rye	Mineral fertilizer		N 34	100 kg
	10.May	(*Secale cereale L.*)	Herbicide	Sekator 375 OD	amidosulfuron /100 g l^{-1}), iodosulfuron (25 g l^{-1})	0.15 l
	15.May		Mineral fertilizer		N 34	100 kg
2008	8. May	potato (*Solanum tuberosum L.*)	Mineral fertilizer		NPK 10:10:20 rimsulfuron 250 g kg^{-1}	600 kg
	19. June		Herbicide	Titus 25 DF		50 g
	19. June		Fungicide	Ridomil Gold MZ 68 WG	metalaxyl (40 g kg^{-1}), mancoceb (640 g kg^{-1})	2.5 kg
	4. July		Fungicide	Shirlan	fluazinam (500 g l^{-1})	0.7 l
	11. July		Fungicide	Shirlan	fluazinam (500 g l-^{1})	0.3 l
	24.July		Fungicide + Insecticide	Ranman + Danadim 40 EC	Cyazofamid (400 g $l^{-1)}$ + dimethoate 400 g l^{-1}	0.2 + 0.5 l
2009	28.April	oat (*Avena sativa L.*)	Mineral fertilizer		NPK 24:6:12	300 kg
	30.May		Herbicide	Sekator 375 OD	amidosulfuron /100 g l^{-1}), iodosulfuron (25 g l^{-1})	0.15 l
2010	28.April	barley (*Hordeum vulgare L.*) with	Mineral fertilizer		NPK 24:6:12	200 kg
	31. May	undersown red clover (*Trifolium pratense L.*)	Herbicide	MCPA	MCPA (750 g l^{-1})	0.9 kg

Table.2 Soil microbial biomass (C_{mic}), nitrogen (N_{mic}), C_{mic}/N_{mic} ratios mean values, soil basal respiration, metabolic quotient CO_2 (qCO_2), dehydrogenase (DHA) and alkaline phosphomonesterase (APA) activity mean values of treatments in 2010

Treatment[a]	Cmic (µg CO2-C g-1 soil)	N_{mic} (µg N g-1 soil)	$Cmic/N_{mic}$ ratio	Basal respiration 164 h (µg CO2-C g-1soil h-1)	qCO2 (µg CO2-C mg Cmic h-1)	Dehydrogenase (µg TPF g-1 soil h-1)	Phosphomonesterase (Nitrophenol µg g-1 soil h-1)
ORGFYM	192.12a	34.23[a]	5.62[a]	0.49[a]	2.58[a]	4.50[a]	241.92[a]
ORGGRM	181.89a	32.29[a]	5.63[a]	0.46[a]	2.52[a]	3.88[b]	196.21[b]
CONFYM	180.11a	33.61[a]	5.35[a]	0.48[a]	2.69[a]	3.73[b]	255.96[a]

Notes. [a]ORGFYM – organic with green and cattle manure; ORGGRM – organic with green manure; CONFYM – conventional with green and cattle manure, mineral fertilizers and pesticides. Different letters behind the mean values (n=4) indicate significant differences ($p<0.05$) in a category.

Fusarium spp. Fusarium species are ubiquitous in soil and are important worldwide plant pathogens (Domsch and Gams, 1970). Fusaria exist in soil as colonizers of living plants or plant residues within the soil or adjacent to the soil surface (Burgess, 1981). The lowest *Fusarium* spp. abundance occurred in spring and highest in fall (Table 3). The main reason for this is that in September there was lot of rainfall, which contributed to the development of *Fusarium* spp. (Figure 1). Also McMullen (1997) states that for the Fusarium population frequent rainfall and high humidity are favorable. Between the treatments no difference was observed (Table 3).

Actinomycetes

The actinomycetes comprise more than 30% of the total population of microorganisms in soil; however, their biomass contribution is variable and much less than that of fungi (Kuster. 1968; Gray, Williams, 1971). In nature, they play an important role in the cycling of organic compounds and have also been associated with soil organic matter production.

The abundance of actinomycetes was in tretments similar and ranged from 0.73 to 1.40 x 10^6 (CFUs, Table 3). Also Frey *et al*., (1999) and Beare *et al*., (1992) found that the total actinomycete communities were affected only minimally by tillage regime and not at all by nitrogen fertilization. Soil organic matter content, pH and moisture also failed to influence actinomycete communities in Western Australian soils (Keast , Tonkin, 1983).

Denitrifying bacteria

Denitrification is mainly sustained by denitrifying bacteria, although the ability of denitrification is also found in certain fungi (Zumft, 1997).

Figure.1 Total precipitation (mm) and average air temperature (°C) decadely during the growing season 2010

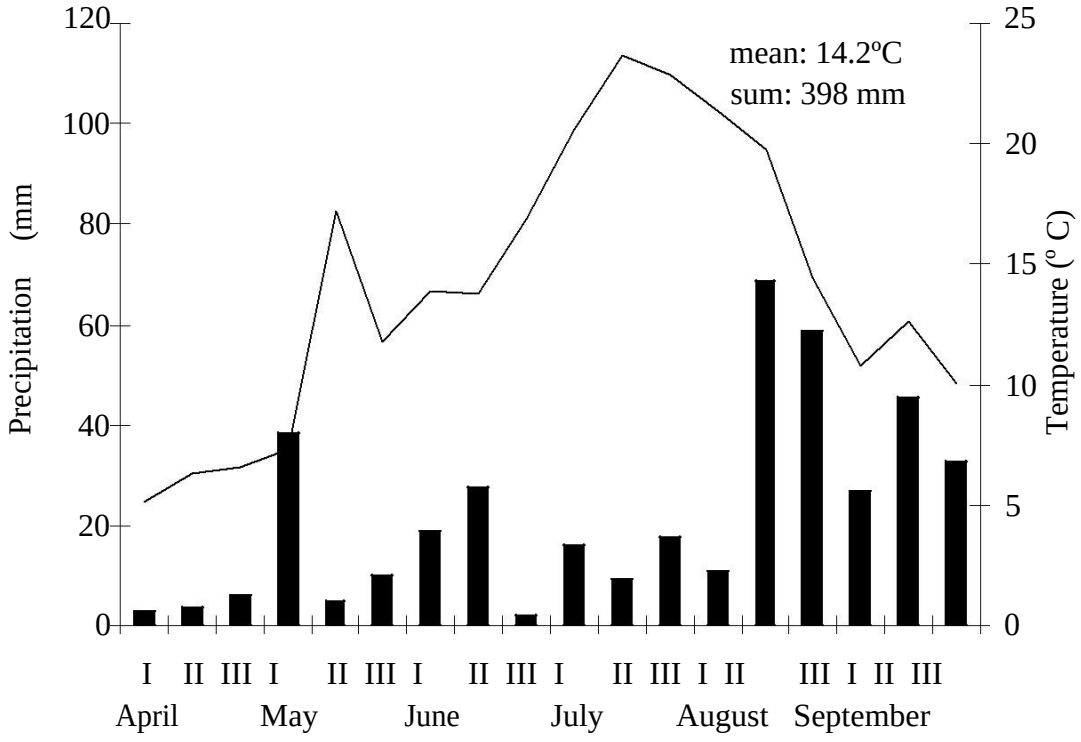

Table.3 Mean values (CFU g^{-1} dry soil) of treatments, sampling dates and their interaction in 2010

Particulars	pH	Total number of bacteria 10^6	Molds	Yeasts	Mesophilic bacteria	Fusarium	Actino mytcetes	Denitri-fying	Nitri-fying	Azoto-bacteria	Cellulose decom-
	(H_2O)		10^4	10^3	10^5	10^3	10^6	bac. 10^5	bac. 10^4	10	posers 10^3
Treatment	7.18[a]	9.75[a]	16.12[a]	1.72[a]	3.90[a]	5.82[a]	1.06[a]	3.17[a]	3.13[a]	9.50[a]	3,26[a]
ORGFYM											
ORGGRM	7.03[b]	8.06[a]	11.66[a]	2.22[a]	3.01[a]	5.58[a]	1.00[a]	4.53[a]	1.87[b]	3.93[a]	2,38[a]
CONFYM	6.68[c]	8.09[a]	9.41[a]	6.68[a]	3.92[a]	5.56[a]	1.15[a]	1.56[a]	3.00[a]	13.17[a]	2,03[a]
Sampling date	6.97[b]	5.87[b]	5.03[b]	0.33[b]	5.38[a]	4.77[b]	1.19[a]	5.21[a]	2.43[b]	3.10[a]	2.40[a]
Spring											
Fall	7.07[a]	11.40[a]	19.77[a]	6.75[a]	1.84[b]	6.53[a]	0.95[a]	0.96[a]	2.90[a]	14.63[a]	2.71[a]
Treatment x Sampling											
date	7.1[ab]	7.07[bc]	5.54[b]	0.00[a]	5.74[a]	4.70[c]	1.40[a]	5.74[a]	2.75[a]	3.78[a]	3.44[a]
ORGFYM, spring											
ORGFYM, fall	7.27[a]	12,43[a]	26,7[a]	3,24[a]	2.06[b]	6.94[a]	0.73[a]	0.59[a]	3.50[a]	15.22[a]	3.09[a]
ORGGRM, spring	6.97[bc]	4.21[c]	4.56[b]	0.20[a]	4.70[a]	4.51[c]	1.12[a]	7.22[a]	1.40[b]	4.15[a]	1.83[a]
ORGGRM, fall	7.1[ab]	11.91[a]	18.77[a]	4.44[a]	1.32[b]	6.66[a]	0.88[a]	1.84[a]	2.34[ab]	3.71[a]	2.92[a]
CONFYM, spring	6.53[d]	6.33[c]	4.99[b]	0.78[a]	5.7[a]	5.11[bc]	1.05[a]	2.68[a]	3.13[a]	1.37[a]	1.93[a]
CONFYM, fall	6.83[c]	9.85[ab]	13.83[ab]	12.57[a]	2.15[b]	6.00[ab]	1.25[a]	0.44[a]	2.86[a]	24.97[a]	2.13[a]
Model effects											
Treatment	<.0001	0.0532	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.0008	n.s.	n.s.
Sampling date	0.0003	<.0001	<.0001	0.0456	<.0001	<.0001	n.s.	n.s.	0.0477	n.s.	n.s.
Treatment x Sampling date	n.s.	0.0366	n.s.	n.s.	n.s.	0.0155	n.s.	n.s.	n.s.	n.s.	n.s.

Notes. [a]ORGFYM – organic with green and cattle manure; ORGGRM – organic with green manure; CONFYM – conventional with green and cattle manure, mineral fertilizers and pesticides. Different letters behind the mean values (n=3) indicate significant differences ($p < 0.05$) in a category. Significances of model effects ($p > F$) are indicated. For significant model effects a post hoc Tukey HSD test was performed to compare mean values. n.s. – Not significant

Denitrifying bacteria reduce nitrate (NO_3^-) to nitrous oxide (N_2O) or to nitrogen gas (N_2). With the ability to degrade organic matter, denitrifying bacteria play a crucial function in reducing organic carbon, thereby reducing nitrate in the wastewater and soils (Hallin and Pell, 1998; Pai *et al*., 1999; Song *et al*., 2000). Factors regulating denitrification rates are low O_2 partial pressure, available NO_3 to serve as an oxidant, and organic C as an energy source for heterotrophic bacteria (Williams *et al*., 1992).

The treatments had unclear effect on the abundance of denitrifying bacteria because of large fluctuations between the replications (Table 3). Although there was tendency for higher level in organic treatments. The lower number of denitrifying bacteria in CONFYM treatment suggests that they are sensitive to pesticides, although in this year in CONFYM treatment only once the herbicides was used. The denitrifying activity is often used for testing the effects of the pesticides because of their sensitivity to environmental toxicants.

Nitrifying bacteria

Nitrifying bacteria are responsible for the biological oxidation of ammonia. These bacteria are chemolithotrophs, obtaining chemical energy from the oxidation process. This energy is used to elaborate organic compounds from carbon dioxide.

Nitrifying bacteria usually occur in small numbers in upper layers of sediments as they are obligate aerobes (Kolwzan et. al 2006). The abundance of nitrifying bacteria was higher in CONFYM (3.00 x 10^4 CFUs) and ORGFYM (3.13 x 10^4 CFUs) treatments, where the solid cattle manure was used and lower in ORGGRM treatment (1.87 x 10^4 CFUs), where only the green manure was used (Table 3).

The abundance of nitrifying bacteria was greatest in fall (2.90 x 10^4 CFUs). This could also have been caused by the uniform rainfall in September, which was favorable on the development of nitrifying bacteria (Table 3, Figure 1). Nitrification is favored at moderate pH and in well-aerated soils, but declines as soils become very dry. The temperature response of nitrification is approximately with an optimum between 20°C and 35°C. The decline at higher temperatures may be partially due to increased biological O_2 consumption (Grundmann *et al*., 1995; Parton *et al*., 2001; Avrahami *et al*., 2003).

Azotobacteria

Azotobacter is a bacterium that can fix atmospheric nitrogen into the soil without the aid of a legume. It has beneficial effects on plant yields, due to the increase of fixed nitrogen content in soil (Zahir *et al*., 1996; Pandey *et al*., 1998).

Fluctuations between the replications were extreme and significant differences between the treatments did not occur (Table 3). During the study, their numbers ranged between 1.37 and 24.97 x 10 (CFUs). However, the results of the analysis in spring and fall showed tendency of greater abundance in fall then the amount of precipitations was significantly higher as in spring (Figure 1).

Cellulose decomposers

The main cellulose utilizing species are the aerobic and anaerobic hemophilic bacteria, filamentous fungi, basidiomycetes, thermophilic bacteria and actinomycetes (Wright, 2003). Mendelssohn *et al*., (1999) note that the soil moisture, temperature as well as fertility, oxygen, and pH are the important extrinsic abiotic variables affect decomposition rate. Our previous studies have shown higher abundance of cellulose

decomposer in the treatments, where the cattle manure was used (Edesi *et al*., 2012). In the present study because fluctuations between the replications the positive effect of manure was not as clear. However, the similar tendency was still noticeable (Table 3).

Conclusion

The aim of our study was to assess the effect of fertilization on soil microbial activity, plate count microorganisms and on soil pH in organic and conventional farming conditions. On the bases of results from present and previous study (Edesi *et al*. 2012), we can conclude that the use of organic fertilizers such as animal manure in addition to the legumes as green manure encourages the microbial activities in the soil. Legumes alone are not sufficient to maintain or increase the soil microbial activity. Therefore, although the green manuring is considered to be an important management practice in organic cultivation to support soil microbial activity and the abundance of microbes in different microbial communities it is important to use other organic fertilizers such as animal manure in addition to green manure.

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