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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Abstract

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 m²) 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 ø 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 (H₂O) was measured according to the standard ISO 10390:2005. Soil microbial biomass C (Cmic) and N (Nmic) 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 K₂SO₄ 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 in fumigated samples – N 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 CO₂ 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 CO₂-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 CO₂ (qCO₂) 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 qCO2 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 qCO2 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, qCO2 values enhanced and the carbon is lost. High qCO2 values indicate stress (Fliessbach et al., 1994). An increased qCO2 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 qCO2 to less availability of soil nutrients, whereas Pascual et al. (1997) indicated that soil under organic practices had a small value of qCO2 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**

Yeast 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

<table>
<thead>
<tr>
<th>Year</th>
<th>Date</th>
<th>Crop</th>
<th>Chemical category</th>
<th>Commercial name</th>
<th>Active ingredient/plant nutrient</th>
<th>Hectare amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>9. April</td>
<td>winter rye</td>
<td>Mineral fertilizer</td>
<td>Sekator 375 OD</td>
<td>N 34 amidosulfuron (100 g l⁻¹), iodosulfuron (25 g l⁻¹)</td>
<td>100 kg</td>
</tr>
<tr>
<td></td>
<td>10. May</td>
<td>(Secale cereale L.)</td>
<td>Herbicide</td>
<td></td>
<td>N 34</td>
<td>0.15 l</td>
</tr>
<tr>
<td></td>
<td>15. May</td>
<td></td>
<td>Mineral fertilizer</td>
<td></td>
<td></td>
<td>100 kg</td>
</tr>
<tr>
<td>2008</td>
<td>8. May</td>
<td>potato (Solanum tuberosum L.)</td>
<td>Mineral fertilizer</td>
<td></td>
<td>NPK 10:10:20 rimsulfuron 250 g kg⁻¹</td>
<td>600 kg</td>
</tr>
<tr>
<td></td>
<td>19. June</td>
<td></td>
<td>Mineral fertilizer</td>
<td>Titus 25 DF</td>
<td></td>
<td>50 g</td>
</tr>
<tr>
<td></td>
<td>19. June</td>
<td></td>
<td>Herbicide</td>
<td>Ridomil Gold</td>
<td>metaxal (40 g kg⁻¹), mancoceb (640 g kg⁻¹)</td>
<td>2.5 kg</td>
</tr>
<tr>
<td></td>
<td>4. July</td>
<td></td>
<td>Fungicide</td>
<td>Shirlan</td>
<td>fluazinam (500 g l⁻¹)</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>11. July</td>
<td></td>
<td>Fungicide</td>
<td>Shirlan</td>
<td>fluazinam (500 g l⁻¹)</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>24. July</td>
<td></td>
<td>Fungicide</td>
<td>Shirlan +</td>
<td>Cyanofamin (400 g l⁻¹) + dimethoate 400 g l⁻¹</td>
<td>0.2 +</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Insecticide</td>
<td>Danadim 40 EC</td>
<td></td>
<td>0.51</td>
</tr>
<tr>
<td>2009</td>
<td>28. April</td>
<td>oat (Avena sativa L.)</td>
<td>Mineral fertilizer</td>
<td>Sekator 375 OD</td>
<td>NPK 24:6:12 amidosulfuron (100 g l⁻¹),</td>
<td>300 kg</td>
</tr>
<tr>
<td></td>
<td>30. May</td>
<td></td>
<td>Herbicide</td>
<td></td>
<td>iodosulfuron (25 g l⁻¹)</td>
<td>0.15 l</td>
</tr>
<tr>
<td></td>
<td>31. May</td>
<td>undersown red clover (Trifolium pratense L.)</td>
<td>Herbicide</td>
<td>MCPA</td>
<td>MCPA (750 g l⁻¹)</td>
<td>0.9 kg</td>
</tr>
</tbody>
</table>

### Table 2: Soil microbial biomass (Cmic), nitrogen (Nmic), Cmic/Nmic ratios mean values, soil basal respiration, metabolic quotient CO₂ (qCO₂), dehydrogenase (DHA) and alkaline phosphomonesterase (APA) activity mean values of treatments in 2010

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cmic (µg CO2-C g⁻1 soil)</th>
<th>Nmic (µg N g⁻1 soil)</th>
<th>Cmic/Nmic ratio</th>
<th>Basal respiration 164 h (µg CO2-C g⁻1 soil h⁻1)</th>
<th>qCO2 (µg CO2-C mg Cmic h⁻1)</th>
<th>Dehydrogenase (µg TPF g⁻1 soil h⁻1)</th>
<th>Phosphomonesterase (Nitrophenol µg g⁻1 soil h⁻1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORGFYM</td>
<td>192.12a</td>
<td>34.23a</td>
<td>5.62a</td>
<td>0.49a</td>
<td>2.58a</td>
<td>4.50a</td>
<td>241.92a</td>
</tr>
<tr>
<td>ORGGRM</td>
<td>181.89a</td>
<td>32.29a</td>
<td>5.63a</td>
<td>0.46a</td>
<td>2.52a</td>
<td>3.88b</td>
<td>196.21b</td>
</tr>
<tr>
<td>CONFYM</td>
<td>180.11a</td>
<td>33.61a</td>
<td>5.35a</td>
<td>0.48a</td>
<td>2.69a</td>
<td>3.73b</td>
<td>255.96a</td>
</tr>
</tbody>
</table>

Notes. aORGFYM – 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 treatments similar and ranged from $0.73 \text{ to } 1.40 \times 10^6$ (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

![Figure 1](image-url)
Table 3: Mean values (CFU g⁻¹ dry soil) of treatments, sampling dates and their interaction in 2010

<table>
<thead>
<tr>
<th>Particulars</th>
<th>pH (H₂O)</th>
<th>Total number of bacteria 10⁶</th>
<th>Molds</th>
<th>Yeasts</th>
<th>Mesophilic bacteria</th>
<th>Fusarium</th>
<th>Actinomycetes</th>
<th>Denitrifying</th>
<th>Nitrifying</th>
<th>Azotobacteria</th>
<th>Cellulose decomposers 10³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>ORGFYM</td>
<td>7.18⁻</td>
<td>9.75⁻</td>
<td>16.12⁻</td>
<td>1.72⁻</td>
<td>3.90⁻</td>
<td>5.82⁻</td>
<td>1.06⁻</td>
<td>3.17⁻</td>
<td>3.13⁻</td>
<td>9.50⁻</td>
<td>3.26⁻</td>
</tr>
<tr>
<td>ORGRGM</td>
<td>7.03⁻</td>
<td>8.06⁻</td>
<td>11.66⁻</td>
<td>2.22⁻</td>
<td>3.01⁻</td>
<td>5.58⁻</td>
<td>1.00⁻</td>
<td>4.53⁻</td>
<td>1.87⁻</td>
<td>3.93⁻</td>
<td>2.38⁻</td>
</tr>
<tr>
<td>CONFYM</td>
<td>6.68⁻</td>
<td>8.09⁻</td>
<td>9.41⁻</td>
<td>6.68⁻</td>
<td>3.92⁻</td>
<td>5.56⁻</td>
<td>1.15⁻</td>
<td>1.56⁻</td>
<td>3.00⁻</td>
<td>13.17⁻</td>
<td>2.03⁻</td>
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<tr>
<td>Sampling date</td>
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<td>Spring</td>
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<td>Treatment x Sampling date</td>
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<tr>
<td>ORGFYM, spring</td>
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<td></td>
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<tr>
<td>ORGFYM, fall</td>
<td>7.27⁻</td>
<td>12.43⁻</td>
<td>26.7⁻</td>
<td>3.24⁻</td>
<td>2.06⁻</td>
<td>6.94⁻</td>
<td>0.73⁻</td>
<td>0.59⁻</td>
<td>3.50⁻</td>
<td>15.22⁻</td>
<td>3.09⁻</td>
</tr>
<tr>
<td>ORGRGM, spring</td>
<td>6.97⁻</td>
<td>4.21⁻</td>
<td>4.56⁻</td>
<td>0.20⁻</td>
<td>4.70⁻</td>
<td>4.51⁻</td>
<td>1.12⁻</td>
<td>7.22⁻</td>
<td>1.40⁻</td>
<td>4.15⁻</td>
<td>1.83⁻</td>
</tr>
<tr>
<td>ORGRGM, fall</td>
<td>7.1⁻</td>
<td>11.91⁻</td>
<td>18.77⁻</td>
<td>4.44⁻</td>
<td>1.32⁻</td>
<td>6.66⁻</td>
<td>0.88⁻</td>
<td>1.84⁻</td>
<td>2.34⁻</td>
<td>3.71⁻</td>
<td>2.92⁻</td>
</tr>
<tr>
<td>CONFYM, spring</td>
<td>6.53⁻</td>
<td>6.33⁻</td>
<td>4.99⁻</td>
<td>0.78⁻</td>
<td>5.7⁻</td>
<td>5.11⁻</td>
<td>1.05⁻</td>
<td>2.68⁻</td>
<td>3.13⁻</td>
<td>1.37⁻</td>
<td>1.93⁻</td>
</tr>
<tr>
<td>CONFYM, fall</td>
<td>6.83⁻</td>
<td>9.85⁻</td>
<td>13.83⁻</td>
<td>2.15⁻</td>
<td>6.00⁻</td>
<td>1.25⁻</td>
<td>0.44⁻</td>
<td>2.86⁻</td>
<td>24.97⁻</td>
<td>2.13⁻</td>
<td></td>
</tr>
<tr>
<td>Model effects</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>&lt;.0001</td>
<td>0.0532</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>0.0008</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Sampling date</td>
<td>0.0003</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>0.0456</td>
<td>&lt;.0001</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>0.0477</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Treatment x Sampling date</td>
<td>n.s.</td>
<td>0.0366</td>
<td>n.s.</td>
<td>n.s.</td>
<td>0.0155</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
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<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Notes: ORGFYM – organic with green and cattle manure; ORGRGM – 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 ($\text{NO}_3^-$) to nitrous oxide ($\text{N}_2\text{O}$) or to nitrogen gas ($\text{N}_2$). 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 $\text{O}_2$ partial pressure, available $\text{NO}_3^-$ 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 ($3.00 \times 10^4$ CFUs) and ORGFYM ($3.13 \times 10^4$ CFUs) treatments, where the solid cattle manure was used and lower in ORGGRM treatment ($1.87 \times 10^4$ CFUs), where only the green manure was used (Table 3).

The abundance of nitrifying bacteria was greatest in fall ($2.90 \times 10^4$ 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 $20^\circ$C and $35^\circ$C. The decline at higher temperatures may be partially due to increased biological $\text{O}_2$ 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 $24.97 \times 10^4$ (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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**References**


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