Soil microbial communities and dehydrogenase activity depending on farming systems

M. Järvan, L. Edesi, A. Adamson, T. Võsa

Department of Agrotechnology, Estonian Crop Research Institute, Saku, Estonia

ABSTRACT

The aim of study was to compare the effect of farming methods on soil microbial communities and dehydrogenase activity (DHA). During 2008–2013, in the five-field crop rotation the following treatments were carried out: ORG – organic; ORGFYM – organic with cattle manure; CONFYM – conventional (cattle manure, mineral fertilizers and pesticides were used). From the treatments soil samples in three replicates were taken for microbiological tests in September yearly. Total bacteria and cellulose decomposing bacteria were determined as a number of colony forming units per g of dry soil. Soil DHA was determined in accordance with Tabatabai (1982). Solid cattle manure applied in the ORGFYM rotation increased significantly ($P < 0.05$) the number of total bacteria and cellulose decomposing bacteria (by 19.4% and 45.3%, respectively), and DHA by 22.7%. There appeared no clear and significant differences in effects between ORGFYM and CONFYM treatments, as an average of experiment period. In some cases, the use of certain pesticides in CONFYM treatment significantly affected the microbe numbers and soil DHA.

Keywords: organic and conventional treatments; cattle manure; total bacteria; cellulose decomposing bacteria; pesticides

Soil microorganisms participate in the processes that are crucial for long-term sustainability of agricultural systems (Nannipieri et al. 2003). In organic systems, plant production depends primarily on nutrient cycling in soils that are controlled by microbes and soil enzymes (Monokrousos et al. 2006, Karaca et al. 2011).

As is stated by many researchers, different farming systems may change soil parameters especially soil microorganisms and enzymes (Monokrousos et al. 2006, Fliessbach et al. 2007, Karaca et al. 2011). In most cases the authors have mentioned that soil microbial diversity and abundance, and soil enzymatic activity were significantly greater in the organic management compared with the conventional one. However, Bowles et al. (2014) showed that differences in organic management have strongly influenced soil enzymes activity, but without a major effect on microbial communities. As mentioned by Shannon et al. (2002), studies of the soils microbial status managed under organic and conventional regimes have produced conflicting evidence of whether there are distinct differences in the microbiological parameters which may be attributed to management practice. According to their research, differences in microbial communities in soils under different management were subtle rather than dramatic, and many of the measured parameters showed no consistently significant differences (Shannon et al. 2002).

Dehydrogenase is an enzyme that occurs in all viable microbial cells. These enzymes function as a measurement of the metabolic state of soil microorganisms (Watts et al. 2010). Dehydrogenase activity (DHA) is one of the most adequate, important and one of the most sensitive bioindicators, relating to soil fertility (Wolinska and Stepniewska 2012). Its activity depends from the same factors, which influence on microorganisms abundance and activity. Besides, it is well known that pesticides have inhibiting effects on DHA (Karaca et al. 2011, Wolinska and Stepniewska 2012).

Two hypotheses were set in our research (1) applying of cattle manure in organically managed crop rotation enhances soil microbial abundance and enzymatic activity, and (2) there are no clear and significant differences between organic and moderate-level conventional farming impacts on the number of soil microorganisms and DHA; but in certain cases pesticides may have a disadvantageous impact on the soil microbial life. The current work is focused on the total bacteria, cellulose decomposing bacteria and soil DHA depending on the organic and conventional farming methods.

MATERIAL AND METHODS

The field experiment was performed in Central-Estonia at Olustvere (58°33'N, 25°34'E) during 2008–2013. The soil type was sandy loam Albeluvisol according to the WRB (2006) classification. The mean characteristics of the soil were as follows: C_{org} 1.5–1.6%, pH_{KCl} 6.1, $P_{Mehlich\ III}$ 195–210 mg/kg, $K_{Mehlich\ III}$ 144–180 mg/kg. In 2007, the five-field crop rotation was established as follows: winter rye, potato, oats, barley with undersowing, red clover. The fields (1.2 ha) were divided into three equal parts (0.4 ha) between the cultivation methods. The following treatments were carried out: organic without manure (ORG); organic with cattle manure (ORGFYM); and conventional (CONFYM) – cattle manure, mineral fertilizers and pesticides were used. In all treatments, red clover as green manure and mouldboard ploughing as tillage method was used. Crop residues were left on the field. In the ORGFYM and CONFYM treatments for potato cattle manure (60 t/ha) was applied prior to ploughing. In the CONFYM treatment, mineral fertilizers were applied at the following rates: for potato – N 60, P 60, K 120 kg/ha; oats – N 72, P 18, K 36 kg/ha; barley with undersown clover – N 48, P 12, K 24 kg/ha; rye – N 15, P 30, K 75 kg/ha in fall and N 34 kg/ha twice in spring. In the CONFYM treatment the weeds were controlled using the following herbicides and doses: oats – Sekator 0.15 L/ha, barley with undersown clover – MCPA 0.9 kg/ha, rye – Mustang Forte 0.7 L/ha, potato – Titus 50 g/ha (in 2008) and Mistral 0.5 kg/ha (in 2013). In 2008, potato was treated with fungicides four times to control late blight (Ridomil Gold 2.5 kg/ha, Shirlan 0.7 and 0.3 kg/ha, and Ranman 0.2 L/ha) and once with insecticide Danadim 0.5 L/ha. In 2013, to potato the fungicides were applied at three times (Ridomil Gold 2.5 kg/ha, Shirlan 0.3 and 0.3 kg/ha) and once the insecticide Fastac 0.3 L/ha.

Soils for microbiological tests discussed in the present paper were sampled from the crop rotation field No 2. In September, from each treatment soil samples in three replicates were taken from the 0–20 cm layer. They were examined for seven different groups of microorganisms. In the current work total bacteria and cellulose decomposing bacteria are discussed. Microbiological counts were expressed as a number of colony forming units (CFUs) per g of dry soil. Plate Count Agar was used for isolation of total bacteria at 30°C for 72 h (NMKL No 86, 1999 and ICC No. 125, 1978). Cellulose decomposing bacteria were identified on Hutchinson culture medium. DHA was measured in accordance with Tabatabai (1982). All results were based on three soil replicates. The data were analyzed by ANOVA. The Tukey-Kramer honestly significant difference (*HSD*) test was used, using the software JMP 5.0.1.2 (SAS, 2002 JMP; SAS Institute, Cary, USA).

RESULTS AND DISCUSSION

Microbial communities. During six-year experiment period, total bacteria and cellulose decomposing bacteria were determined in four years in September in the treated soils (Table 1). In 2010, in both organic treatments the numbers of total bacteria were larger than in the other years. Their number reached up to 12×10^6 CFUs per 1 g dry soil. We suppose, on one hand, that such great increase in the bacterial community took place owing to the viable growth of clover sown under barley. On the other hand, the growth period in that year was warmer than the many-years average, whereat the end of summer was extraordinary warm. According to Pettersson and Bååth (2003), temperature is one of the most important environmental factors affecting soil bacterial community.

In 2008, 2009 and 2013, there appeared the significant ($P < 0.05$) differences in the numbers of total bacteria between the ORG and ORGFYM treatments. Cattle manure applied in the ORGFYM crop rotation had a good direct effect and aftereffect both on the bacterial community and on the crop yields (increasing potato yield from 19.2 to 30.9 t/ha, and oat yield from 2.2 to 2.9 t/ha). The application of cattle manure increased the number of total bacteria by 19.4%, and the number of cellulose decomposing bacteria by 45.3%, as an average of four years. Our results are in accordance with

Table 1. The influence of farming methods on the number of soil bacteria (CFU/g dry soil) and DHA (TPF μg/g/h)

	Treatment	2008 potato	2009 oats	2010 barley + clover	2012 rye	2013 potato	Average
Total bacteria × 10^{6}	ORG	8.53^{b}	6.07^{b}	11.91^{a}	–	7.07^{b}	8.40^{b}
	ORGFYM	10.80^{a}	7.93^{a}	12.43^{a}	–	8.95^{ab}	10.03^{a}
	CONFYM	8.55^{b}	7.73^{a}	9.85^{b}	–	10.60^{a}	9.18^{ab}
Cellulose decomposing bacteria × 10^{3}	ORG	1.64^{b}	3.12^{b}	2.92^{ab}	–	1.76^{b}	2.36^{b}
	ORGFYM	3.85^{a}	3.25^{b}	3.09^{a}	–	3.54^{a}	3.43^{a}
	CONFYM	1.76^{b}	4.79^{a}	2.13^{b}	–	3.84^{a}	3.13^{a}
DHA	ORG	–	–	3.88^{b}	2.33^{b}	2.78^{b}	3.00^{b}
	ORGFYM	–	–	4.50^{a}	2.40^{b}	4.15^{a}	3.68^{a}
	CONFYM	–	–	3.73^{b}	3.32^{a}	3.25^{b}	3.43^{ab}

CFU – colony forming units per 1 g dry soil; TPF – triphenylformazan; DHA – dehydrogenase activity; ORG – organic without manure; ORGFYM – organic with cattle manure; CONFYM – conventional (cattle manure, mineral fertilizers and pesticides). Different letters behind the mean values indicate significant differences ($P < 0.05$) in a category

other researchers (Parham et al. 2002, Fliessbach et al. 2007, Scherer et al. 2011) who demonstrated positive effect of manure to the abundance of soil microorganisms.

Comparison of the ORGFYM and CONFYM treatments revealed that in 2008 and 2010 the numbers of total bacteria in the soil of CONFYM treatment were significantly ($P < 0.05$) lower than in the soil of ORGFYM treatment. We assume that it was a consequence of the pesticide application. In 2008, potato was repeatedly treated with fungicides and once with insecticide Danadim (active ingredient – dimethoate). As mentioned by Haleem et al. (2013), dimethoate significantly ($P < 0.05$) decreased the counts of total bacteria in laboratory conditions. In 2010, the weeds were controlled by MCPA. It is liable that this herbicide might noxiously affect on the bacterial communities, because in the soil of CONFYM treatment the numbers of total bacteria and cellulose decomposers decreased by 21% and 31%, respectively, as compared with the ORGFYM treatment. Our results are similar to the results of Schellenberger et al. (2012) who reported about the toxic effect of MCPA on the cellulose degrading bacteria in the water-saturated soil. Such moisture conditions existed in 2010 because during August and September it rained the total of 243 mm.

The toxic effect of pesticides on the cellulose decomposing bacteria probably appeared also in 2008 when their number in the soil of the CONFYM treatment was more than 2 times lower than that of the ORGFYM treatment. In 2009, the number of these bacteria, on the contrary, was significantly highest in the CONFYM treatment. It probably may be explained with the higher amounts of oat roots and straw residues because the grain yield from the CONFYM area (3.9 t/ha) was significantly higher than from the organic plots. The average results of four-year experiment showed that the application of cattle manure in the crop rotation significantly enhanced the abundance of cellulose decomposing bacteria whereas there were no differences between organic and conventional treatments.

Dehydrogenase activity. Dehydrogenase reflects the total range of oxidative activity of soil microflora (Liang et al. 2014). In our study, the DHA in the soils of treatments was measured in September 2010, 2012 and 2013 (Table 1). In 2010, the DHA in the ORGFYM treatment was significantly ($P < 0.05$) higher than in the ORG treatment. It was very likely caused due to the positive aftereffect of cattle manure which was applied at the high rate for the pre-preceding crop, i.e. potato. Also, according to the results of other researchers (Parham et al. 2002, Scherer et al. 2011, Liang et al. 2014), in soils treated with farmyard manure (FYM) the DHA is increasing. In 2012, when rye was grown in the crop rotation, no differences in the DHA were revealed between

ORG and ORGFYM treatments. It can be explained with the lacking aftereffect of FYM because it was applied on this field four years ago. The absence of manure aftereffect was reflected also in the rye yields which were practically equal in both organic treatments (2.8 and 3.0 t/ha). In 2013, potato was grown in the rotation and in manure at the high rate was applied the ORGFYM and CONFYM treatments. In this year, the DHA in the soil of ORGFYM treatment was by 1.5 times higher than in the ORG treatment. Our results are in the same direction with the findings of Watts et al. (2010) and Scherer et al. (2011) which proposed that the treatments with high organic amendments may exhibit the greatest DHA. As an average of the three year experiment, the DHA in ORGFYM treatment was by 22.7% higher than the ORG treatment.

By comparing ORGFYM and CONFYM treatments the results were not in the same direction. In 2010 and 2013, the DHA in the soil of ORGFYM was significantly higher than in the CONFYM treatment. But in 2012, the results were in opposite. The decrease in DHA was probably caused by pesticides applied to the CONFYM treatment. In 2010, the herbicide MCPA very likely affected negatively the soil DHA. It may be concluded also on the ground of the decreasing number of the total bacteria (Table 1) because according to Scherer et al. (2011) and Wolinska and Stepniewska (2012), DHA is directly related to the microbial biomass. Our results are supported also by the research of Wolinska and Stepniewska (2012) who concluded that MCPA herbicide cause toxic effect on soil enzymatic activity.

In 2012, the DHA in the soil of the CONFYM treatment was by 38% higher than in the ORGFYM. In the CONFYM, rye was additionally moderately treated with mineral fertilizer and with normal rate of herbicide. Incidentally, the grain yield in the CONFYM treatment was higher than that in the ORGFYM treatment (4.0 and 3.1 t/ha, respectively). In our experiment, the increase of soil DHA in the CONFYM treatment probably might have been caused by higher yields of conventionally grown rye and preceding crops that left much more straw and root residues in the soil which enhanced enzymatic activity of microbes. According to Karaca et al. (2011), pesticides affect soil enzyme activities in different ways; the application of organic amendments in soils treated with a certain herbicide may result in stimulation of DHA.

In 2013, the DHA in the soil of CONFYM was significantly lower than that of the ORGFYM treatment. Most probably it was a result of an abundant application of pesticides for potato, whereas the amount of active ingredients was 2.365 kg/ha in total. Even though Bünemann et al. (2006) and Banks et al. (2014) have mentioned that herbicides have minimal or no effect on soil microbes, we still think that metribuzin which was applied at the rate 350 g/ha as an active ingredient of herbicide Mistral, might have a disadvantageous effect on the soil microbial and enzymatic activity. Lone et al. (2014) stated that metribuzin at the rate 300 g/ha affected soil bacteria negatively. Also Niemi et al. (2009) showed an inhibiting effect of metribuzin in field tests. As demonstrated by several authors, fungicides and insecticides are much more toxic to a certain microbial groups than herbicides (Bünemann et al. 2006). Thereby, we suppose that the DHA decrease of the CONFYM soil in fall 2013 might be partially caused also by the repeated spraying of potato with fungicides. As proved by Niemi et al. (2009), fluazinam (an active ingredient of the Shirlan used in our experiment) was highly toxic for the soil microorganisms. As an average of the three years, the highest soil DHA appeared in such organic treatment where cattle manure was applied during rotation.

The results of our study allowed making the following conclusions: (1) cattle manure applied in organically managed crop rotation increased the numbers of total bacteria and cellulose decomposing bacteria, and the soil DHA; (2) no clear and significant differences in effect appeared between organically and conventionally managed systems because the use of certain pesticides might disadvantageously affect the soil microorganisms.

REFERENCES

Banks M.L., Kennedy A.C., Kremer R.J., Eivazi F. (2014): Soil microbial community response to surfactants and herbicides in two soils. Applied Soil Ecology, *74*: 12–20.

Bowles T.M., Acosta-Martínez V., Calderón F., Jackson L.E. (2014): Soil enzyme activities, microbial communities, and carbon and nitrogen availability in organic agroecosystems across an intensively-managed agricultural landscape. Soil Biology and Biochemistry, *68*: 252–262.

Bünemann E.K., Schwenke G.D., van Zwieten L. (2006): Impact of agricultural inputs on soil organisms – A review. Soil Research, *44*: 379–406.

Fliessbach A., Oberholzer H.-R., Gunst L., Mäder P. (2007): Soil organic matter and biological soil quality indicators after 21 years of organic and conventional farming. Agriculture, Ecosystems and Environment, *118*: 273–284.

Haleem A.M., Kasim S.A., Al-Timimy J.A. (2013): Effect of some organophosphorous insecticides on soil microorganisms populations under lab condition. World Environment, *3*: 170–173.

Karaca A., Cetin S.C., Turgay O.C., Kizilkaya R. (2011): Soil enzymes as indication of soil quality. In: Shukla G., Varma A. (eds.): Soil Enzymology. Springer-Verlag, Berlin, Heidelberg, 119–148.

Liang Q., Chen H., Gong Y., Yang H., Fan M., Kuzyakov Y. (2014): Effects of 15 years of manure and mineral fertilizers on enzyme activities in particle-size fractions in a North China Plain soil. European Journal of Soil Biology, *60*: 112–119.

Lone A.H., Raverkar K.P., Pareek N. (2014): *In-vitro* effects of herbicides on soil microbial communities. The Bioscan, *9*: 11–16.

Monokrousos N., Papatheodorou E.M., Diamantopoulos J.D., Stamou G.P. (2006): Soil quality variables in organically and conventionally cultivated field sites. Soil Biology and Biochemistry, *38*: 1282–1289.

Nannipieri P., Ascher J., Ceccherini M.T., Landi L., Pietramellara G., Renella G. (2003): Microbial diversity and soil functions. European Journal of Soil Science, *54*: 655–670.

Niemi R.M., Heiskanen I., Ahtiainen J.H., Rahkonen A., Mäntykoski K., Welling L., Laitinen P., Ruuttunen P. (2009): Microbial toxicity and impacts on soil enzyme activities of pesticides used in potato cultivation. Applied Soil Ecology, *41*: 293–304.

Parham J.A., Deng S.P., Raun W.R., Johnson G.V. (2002): Long-term cattle manure application in soil. I. Effect on soil phosphorus levels, microbial biomass C, and dehydrogenase and phosphatase activities. Biology and Fertility of Soils, *35*: 328–337.

Pettersson M., Bååth E. (2003): Temperature-dependent changes in the soil bacterial community in limed and unlimed soil. FEMS Microbiology and Ecology, *45*: 13–21.

Schellenberger S., Drake H.L., Kolb S. (2012): Impairment of cellulose- and cellobiose-degrading soil *Bacteria* by two acidic herbicides. FEMS Microbiology Letters, *327*: 60–65.

Scherer H.W., Metker D.J., Welp G. (2011): Effect of long-term organic amendments on chemical and microbial properties of a luvisol. Plant, Soil and Environment, *57*: 513–518.

Shannon D., Sen A.M., Johnson D.B. (2002): A comparative study of the microbiology of soils managed under organic and conventional regimes. Soil Use and Management, *18*: 274–283.

Tabatabai M.A. (1982): Soil enzymes. In: Page A.L., Miller R.H., Keeney D.R. (eds.): Methods of Soil Analysis, Part 2. American Society of Agronomy and Soil Science Society of America, Madison.

Watts D.B., Torbert H.A., Feng Y., Prior S.A. (2010): Soil microbial community dynamics as influenced by composted dairy manure, soil properties, and landscape position. Soil Science, *175*: 474–486.

Wolinska A., Stepniewska Z. (2012): Dehydrogenase activity in the soil environment. In: Canuto R.A. (ed.): Dehydrogenases. Intech, Rijeka. Available at http://www.ebook3000.com/

Received on May 20, 2014
Accepted on September 17, 2014

Corresponding author:

Dsc. Agr. Malle Järvan, Estonian Crop Research Institute, Department of Agrotechnology,
Teaduse 13, 755 01 Saku, Estonia
e-mail: malle.jarvan@etki.ee