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## Composition of the microbial community in long-term organic and conventional farming systems

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### Abstract

Conventional agriculture has a remarkable influence on nature mainly due to the use of mineral fertilisers and chemical pesticides. Nowadays, organic farming is being increasingly transitioned towards regenerative agriculture. Long-term five-field crop rotation experiment investigated the effect of different fertilisation in organic and conventional farming systems. Soil chemical properties, microbial biomass, and microbial community structure in field trial with winter cover crops (OrgI) and winter cover crops with composted cattle manure (OrgII) as an organic farming system were analysed in comparison with conventional farming system with mineral fertilisers and chemical pesticides (ConvII) or only chemical pesticides without fertilisation (Conv0). Treatments with no extra organic amendments or mineral fertilisers were control for comparison. Two field experiments with different main crops were investigated: potato (pre-crop pea) and spring barley underseeded with red clover (pre-crop potato). All microbial groups (Gram-positive, Gram-negative, *Actinobacteria*, and fungi) were higher in organic farming systems (OrgII and OrgI) compared to conventional system in both treatments (potato and barley). The experiment with potato showed higher values of microbial biomass but little significant differences between fertilisation regardless of farming practice. The experiment with spring barley with red clover showed lower amount of microbial biomass, but biomass by microbial groups (Gram-positive and Gram-negative bacteria, total bacteria, *Actinobacteria*, and fungi) were significantly higher in treatments with organic amendments. The relative abundance of microbial community did not show significant differences between farming systems or fertilisation practices, except for higher abundance of *Actinobacteria* in conventional farming system indicating a significant effect of pesticides on this group. It was found that an organic farming system helps to maintain or increase the abundance of soil microbial community, which is an important part of soil health.

Keywords: long-term field fertility experiment, crop rotation, cover crops, manure, mineral fertilisers, phospholipid fatty acid.

### Introduction

In the European Union (EU), the total area under organic farming was 8.5% (13.8 million hectares) of total agricultural land in 2019 (Eurostat, 2021) and continues to increase due to the European Green Deal (EC, 2019). According to the latest Eurostat (2021) news, Estonia has one of the highest shares of land used for organic farming (22.3%). The European Green Deal is a set of political strategies that directly impact agriculture. According to the Biodiversity Strategy for 2030 (EC,

2020), the goal is to increase organic farming to 25% (compared to current 8%) of all agricultural land area by 2030. Secondly, part of the strategic plan of the Farm to Fork strategy is reducing chemical pesticides by 50% and mineral fertilisers by 20% by 2030. Finally, the EU Climate Target Plan for 2030 aims to reduce greenhouse gas emissions at least by 55% compared with 1990 levels (EC, 2020). All these strategies are directly or indirectly related to organic farming.

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Soil plays an important role in agricultural ecosystems. Soil health is characterised by chemical, physical, and biological properties. Soil conventional management expands agricultural production but impairs several soil functions. For example, the conventional farming system decreases the content of total nitrogen ( $N_{\text{tot}}$ ) and organic carbon ( $C_{\text{org}}$ ), weakens the soil structure, and reduces the biodiversity of soil microorganisms (Edesi et al., 2012; Ereemeev et al., 2020; Massaccesi et al., 2020). On the other hand, the organic farming system with the addition of organic matter improves chemical and biological properties of soil (Edesi et al., 2012; Brust, 2019; Ereemeev et al., 2020), but crop yield usually decreases (Alaru et al., 2014; Massaccesi et al., 2020). Hence, there are many comparative studies of conventional and organic farming, which compare yield and different chemical parameters. However, more and more attention should be paid to physical and biological parameters (Sainju et al., 2021). This supports the changes towards regenerative agriculture and helps adapting to changing environments in the context of climate change. For example, widely used chemical pesticides generally have a long-term inhibitory effect on soil microbial community compared with organic farming (Riedo et al., 2021), but some part of biological component could help to decrease this negative effect.

Several methods of agricultural practices, such as using winter cover crops as catch crop of nutrients and adding manure as organic fertiliser, have been used to sustain soil quality and productivity in organic farming (Pullens et al., 2021). Nitrogen (N) is slowly released in soil by microbes for plant uptake, and mineralisation rate depends on the C:N ratio of used organic material (Brust, 2019). The use of winter cover crops (or catch crops) is a growing trend in agriculture as one possibility for sustainable agriculture. The main objectives are reducing nitrate leaching (Massaccesi et al., 2020), improving N availability and increasing the yield of subsequent crops (Toom et al., 2019; Pullens et al., 2021). In addition, the use of cover crops increases content of soil organic carbon (SOC), which is an important indicator of soil health (Crystal-Ornelas et al., 2021).

Soil microorganisms are an important part of soil development, improvement, and preservation. Anderson (2003) showed a significant relationship between microbial ( $C_{\text{mic}}$ ) and total organic carbon ( $C_{\text{org}}$ ) and, therefore, microbial biomass could be used as soil quality indicators. In addition, it has been shown that soil biological properties are more sensitive indicators of different changes compared to soil chemical and physical properties (Nelson et al., 2009). It is important to analyse relationships between microbial community and agroecosystem management. Long-term field experiments could facilitate the understanding of the relationships between soil microbial health and management practices.

Previous studies of the same long-term five-field crop rotation experiment showed that cover crops and composted cattle manure increased soil microbial hydrolytic activity together with  $N_{\text{tot}}$  and  $C_{\text{org}}$  content in potato cultivation (Ereemeev et al., 2020). However, during the first rotation, the total dry matter yield from organic treatments was significantly smaller than from conventional treatments (Alaru et al., 2014). To clarify the effect of different fertilisation treatments according to farming systems, there is a need for more detailed microbiological studies such as biomass evaluation and estimation of different microbial groups. Detailed information about the soil microbial community help to clarify the positive effect of regenerative agriculture and to adapt changing environmental conditions due to climate change.

The structure and biomass of microbial community could be determined by phospholipid fatty acid (PLFA) analysis (Blight, Dyer, 1959). The PLFA method evaluates only viable microbes and due to that

it is sensitive to the analysis of microbial community in response to treatments compared to different DNA- and RNA-based approaches (Chen et al., 2019).

The aim of this study was to determine the effect of agroecosystem practices on the composition of the microbial community. The hypotheses are: (1) the total microbial biomass in organic farming system is higher compared to the conventional farming; (2) conventional farming system has different structure of microbial community compared to organic farming, and (3) cover crops and main crops have a significant influence on total microbial biomass and community structure.

## Materials and methods

**Sampling site.** The long-term five-field crop rotation experiment with organic and conventional farming systems was started in 2008 at the Department of Crop Science and Plant Biology (58°22' N, 26°40' E), Estonian University of Life Sciences. The long-term crop rotation was as follows: spring barley (*Hordeum vulgare* L.) underseeded with red clover (*Trifolium pratense* L.) → red clover (*Trifolium pratense* L.) → winter wheat (*Triticum aestivum* L.) → field pea (*Pisum sativum* L.) → potato (*Solanum tuberosum* L.). The size of each plot was 60 m<sup>2</sup> with three replicates. Soil type: *Stagnic Luvisol* (WRB, 2014), sandy silt loam. At the beginning of field-trial, mean characteristics of the humus horizon were as follows: pH<sub>KCl</sub> 5.9,  $C_{\text{org}}$  1.1–1.2%,  $N_{\text{tot}}$  0.10–0.12%, P 107–120 mg kg<sup>-1</sup> and K 114–118 mg kg<sup>-1</sup>, with average depth of the humus layer of 30 cm (Reintam, Köster, 2006).

The mean air temperature from autumn to spring was 1.6°C higher than the long-term average (2.3°C). The amount of precipitation during that period was 449 mm, which is higher than the long-term average (434 mm). However, during the sampling period, weather conditions in April were extremely dry: the monthly precipitation was only 4 mm compared to the long-term average of 30 mm.

In this paper, organic and conventional farming systems with five fertilisation treatments (control, OrgI, OrgII, Conv0, and ConvII) are covered. In the organic farming system, cover crops and composted cattle manure were used in OrgII treatment, and only cover crops were used in OrgI one. In the conventional farming system, mineral fertilisers and chemical pesticides were used in ConvII treatment, and only chemical pesticides without mineral fertilisers were used in Conv0 one. Plots that only followed the rotation with no extra fertilisation were control. After harvest, all plant residues remained in the field.

To evaluate the influence of main crops and different fertilisation treatments on soil chemical properties, microbial biomass, and structure, two crops were under observation. The first crop was potato (*Solanum tuberosum* L., cultivar 'Maret'), where the previous crop was pea. The second crop was spring barley (*Hordeum vulgare* L., cultivar 'Anni') with underseeded red clover (*Trifolium pratense* L., cultivar 'Varte'), where previous crop was potato.

**Soil samples.** In spring 2018, eight subsamples for one average sample were taken with a soil drill along the entire length of 0–25 cm. Soil samples were taken from each plot from all treatments. The samples were air-dried and sieved with a 2 mm sieve. Different chemical properties of the soil have been described: pH<sub>KCl</sub>, the content of soil organic carbon (SOC) (%) by Tyurin method, and total nitrogen ( $N_{\text{tot}}$ ) (%) by Kjeldahl method. For microbial community analysis, ten subsamples along the entire length of 0–20 cm were randomly taken from each 60 m<sup>2</sup> plot and pooled together (approx. 0.5 kg). Soil samples were taken with a soil drill in the spring after sowing spring barley with red clover and planting potato tubers in 2019. For phospholipid fatty acid (PLFA)

**Table 1.** Long-term five-field crop rotation experiment of organic (OrgII, OrgI, and control) and conventional (ConvII and Conv0) farming systems

Treatment	Potato	Spring barley with red clover
OrgII	Organic fertiliser: 20 t ha <sup>-1</sup> cattle manure Cover crop: mixture of winter rye and winter oilseed turnip	Organic fertiliser: 10 t ha <sup>-1</sup> cattle manure Cover crop: winter rye
OrgI	Organic fertiliser: cover crop: mixture of winter rye and winter oilseed turnip	Organic fertiliser: cover crop: winter rye
Control	–	–
ConvII	Mineral fertilisers: N <sub>150</sub> P <sub>25</sub> K <sub>95</sub> (ammonium nitrate N34.4 and YaraMila Cropcare 8:11:24) Herbicides: Roundup Gold (a.i. glyphosate) 4.0 l ha <sup>-1</sup> in previous autumn and Titus 50 g ha <sup>-1</sup> in spring Fungicides: Ridomil Gold MZ 68 WG 2.5 kg ha <sup>-1</sup> and Ranman 0.2 kg ha <sup>-1</sup> with activator Ranman 0.15 l ha <sup>-1</sup> Insecticide: Proteus 0.75 l ha <sup>-1</sup>	Mineral fertilisers: N <sub>120</sub> P <sub>25</sub> K <sub>95</sub> (ammonium nitrate N34.4 and YaraMila Cropcare 8:11:24) Herbicides: Roundup Gold (a.i. glyphosate) 4.0 l ha <sup>-1</sup> in previous autumn and Titus 50 g ha <sup>-1</sup> in spring Fungicides: Ridomil Gold MZ 68 WG 2.5 kg ha <sup>-1</sup> and Ranman 0.2 kg ha <sup>-1</sup> with activator Ranman 0.15 l ha <sup>-1</sup>
Conv0	Mineral fertilisers: herbicides: Roundup Gold (a.i. glyphosate) 4.0 l ha <sup>-1</sup> in previous autumn and Titus 50 g ha <sup>-1</sup> in spring Fungicides: Ridomil Gold MZ 68 WG 2.5 kg ha <sup>-1</sup> and Ranman 0.2 kg ha <sup>-1</sup> with activator Ranman 0.15 l ha <sup>-1</sup> Insecticide: Proteus 0.75 l ha <sup>-1</sup>	Mineral fertilisers: herbicides: Roundup Gold (a.i. glyphosate) 4.0 l ha <sup>-1</sup> in previous autumn and Titus 50 g ha <sup>-1</sup> in spring Fungicides: Ridomil Gold MZ 68 WG 2.5 kg ha <sup>-1</sup> and Ranman 0.2 kg ha <sup>-1</sup> with activator Ranman 0.15 l ha <sup>-1</sup>

analysis, soil samples were sieved with a 2 mm sieve and freeze-dried.

*Microbial community composition* was evaluated using PLFA analysis (Frostegård, Bååth, 1996). For PLFA analysis, approximately 4 g of soil were used according to Börjesson and colleagues (1998). The methylated PLFAs (fatty acid methyl esters, FAMES) were quantified by analysis of a Hewlett-Packard 6890 GC System (Agilent Technologies Inc., USA) with a fused silica capillary column (30 m × 0.32 mm). In the splitless mode, 1 µl of samples were injected and results were detected using a flame ionization detector (Agilent Technologies). Identification of the fatty acids (FA) was conducted by retention times in comparison to defined mixture of external standards by gas chromatography-mass spectrometry (GC-MS) analysis. The concentration of FAMES was quantified in relation to methyl nonadecanoate (19:0) (Larodan AB, Sweden) as an internal standard. The oven temperature was raised from 50°C to 110°C at a rate of 30°C min<sup>-1</sup>, from 110°C to 190°C at 1°C min<sup>-1</sup>, and from 190°C to 300°C at 31.4°C min<sup>-1</sup>. Microbial biomass amount was evaluated as nmol g<sup>-1</sup> dry soil; the relative abundance of microbial groups (% mol) was calculated as microbial community structure. The total amount of PLFA was calculated for Gram-positive bacteria as the sum of branched i15:0, a15:0, i16:0, i17:0, and br18:0 FA and for Gram-negative bacteria as the sum of cyclopropane and monounsaturated cy17:0, 16:1ω7c, 16:1ω7t, 18:1ω7c, and cy19:0 FA. The sum of total bacteria represents the sum of Gram-negative and Gram-positive bacteria and 15:0, 17:0, and 18:0 FA. The group of *Actinobacteria* was represented as the sum of 10Me16:0, 10Me17:0, and 10Me18:0 FA. For saprotrophic fungi, the biomarker 18:2ω6 was considered.

*Statistical analysis.* To determine the effect of different main crops and fertilisation in organic and conventional farming systems on soil chemical properties, microbial biomass content, and community structure, analysis of variance (ANOVA) was used. For analysing significant differences between treatments, descriptive statistics: mean, standard deviation (SD), and Tukey-Kramer (HSD) test, were used. To visualise the variation of microbial groups (nmol g<sup>-1</sup> dry soil) across treatments, the principal component analysis (PCA) was used. All analyses were conducted based on software Rstudio, version 1.2.5033 (R Foundation of Statistical Computing, Austria).

## Results and discussion

*Soil chemical properties.* In current study, the effect of the farming systems, fertilisation, and main

crops on soil chemical properties, microbial biomass content, and community composition of the long-term five-field crop rotation experiment is presented. Two-way ANOVA indicated that no significant differences were found between fields with different main crops, and most of the differences were due to fertilisation. Significant differences between fertilisation treatments were found in SOC content ( $p < 0.001$ ), soil acidity (pH) ( $p < 0.05$ ), and C to N ratio ( $p < 0.05$ ) (Table 2).

Organic farming systems with cover crops and cattle manure (OrgI and OrgII) indicated a slight increase in pH compared to the beginning of the field-trial in 2008 (pH = 5.9). On the contrary, the use of mineral fertilisers and chemical pesticides decreased the soil pH. In more detail, after ten years, the average pH ranged from 5.73 in Conv0 to 5.51 in ConvII treatment. The pH in organic farming systems with organic amendments ranged from 5.96 in OrgI to 6.06 in OrgII treatments (Table 2). Control treatments showed intermediate results between organic (OrgI and OrgII) and conventional (Conv0 and ConvII) farming systems. A similar pattern was observed with SOC, where the highest value (1.71%) was in OrgII treatment. Significantly similarly lower values of SOC were found in Conv0 and ConvII treatments. The ratio of C:N in soil between all fertilisation treatments ranged from 12.41 in Conv0 to 13.94 in ConvII treatment. Hence, the ratio of C:N was significantly the highest in ConvII treatment but the lowest in Conv0 one. In conclusion, different fertilisation treatments affect soil chemical properties, mainly pH, SOC, and the C:N ratio.

However, according to the results of current experiment, no significant effect on soil N<sub>tot</sub> content were observed despite different fertilisation in the long-term crop rotation. The absence of significant differences in N<sub>tot</sub> content in spring indicates a strong relationship between external N addition, N uptake by the crops and removal by yield. Results of a previous study (Alaru et al., 2014) has shown that the highest amount of applied N and N recovery percentage was the highest in ConvII treatment and the lowest in the control plots and Conv0 one. In addition, the N<sub>tot</sub> did not differentiate inorganic and organic forms including plant available inorganic forms, decomposed active organic matter, and chemically stable passive humus.

*Soil microbial biomass content.* In total, 28 FAMES were identified and quantified from soil samples taken in spring 2019. Weather conditions and the sampling time have a major influence on soil microbial community and thus on soil chemical properties. Usually, the total amount of PLFA increases over the

**Table 2.** Soil chemical analysis ( $\pm$  SD) between fertilisation treatments in a long-term five-field crop rotation experiment (n = 3)

Treatment	pH <sub>KCl</sub>	Soil organic carbon %	Total nitrogen %	C:N
Control	5.83 ab ( $\pm$ 0.21)	1.53 ab ( $\pm$ 0.06)	0.12 a ( $\pm$ 0.01)	12.84 ab ( $\pm$ 0.44)
OrgI	5.96 ab ( $\pm$ 0.18)	1.53 ab ( $\pm$ 0.13)	0.11 a ( $\pm$ 0.01)	13.42 ab ( $\pm$ 0.53)
OrgII	6.06 a ( $\pm$ 0.18)	1.71 a ( $\pm$ 0.14)	0.12 a ( $\pm$ 0.01)	13.73 ab ( $\pm$ 0.26)
Conv0	5.73 ab ( $\pm$ 0.28)	1.35 b ( $\pm$ 0.07)	0.11 a ( $\pm$ 0.01)	12.41 b ( $\pm$ 1.54)
ConvII	5.51 b ( $\pm$ 0.43)	1.46 b ( $\pm$ 0.12)	0.11 a ( $\pm$ 0.01)	13.94 a ( $\pm$ 0.74)

Note. Control – no fertilisation of winter cover crops, OrgI – a mixture of winter cover crops, OrgII – a mixture of winter cover crops and cattle manure, Conv0 – herbicides, fungicides, and no mineral fertilisation, ConvII – herbicides, fungicides, and NPK fertilisation; different letters indicate significant differences between treatments (Tukey's test,  $p < 0.05$ ).

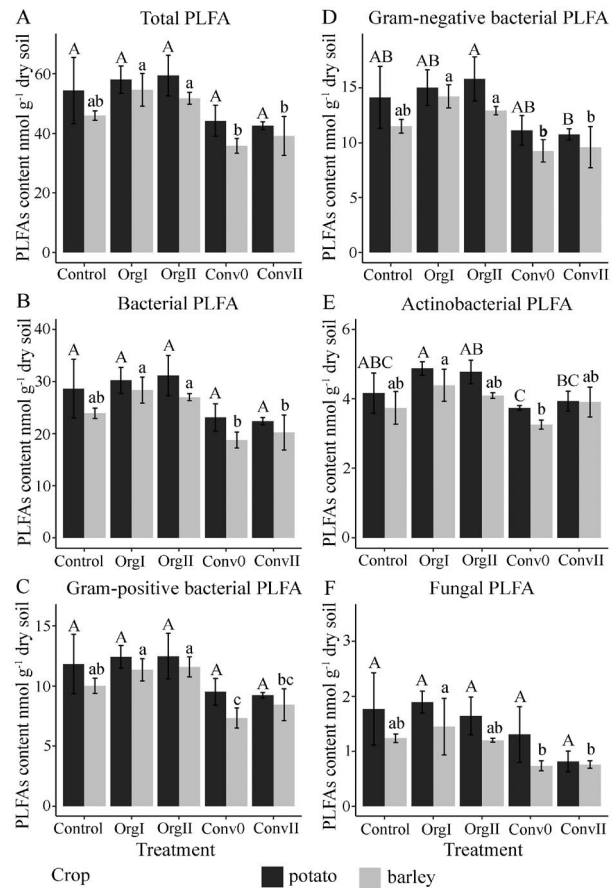
cropping season, especially when the rainfall is heavy (Shahbaz et al., 2020). Another important factor is the time of fertilisation, especially the addition of organic amendments as different sources of carbon to microbes (Toleikiene et al., 2021). To reduce the influence of the main crop and weather conditions on the microbial community during the cropping season, soil samples were taken in spring.

The microbial biomass content was higher (43.64–59.47 nmol g<sup>-1</sup> dry soil) in the experiment with potato, where the pre-crop was pea. Comparing total microbial biomass of the experiment with spring barley, where the pre-crop was potato, the total microbial biomass was noticeably lower (35.83–54.63 nmol g<sup>-1</sup> dry soil) (Figure 1).

The results of current experiment showed that soil microbiology is strongly influenced by pre-crops and that potato as a pre-crop reduces soil microbial biomass. The data of a previous study (Nelson et al., 2009) confirmed the results of current experiment that potato in the rotation system showed a significantly negative effect on soil biological properties, e.g., microbial biomass C, mineralizable C, earthworm abundance, and biomass, but had no effect on soil physical and chemical properties, e.g., bulk density, pH, total SOC, and N. Moreover, the same field trial data showed that potato cultivation negatively affected microbial activity (Eremeev et al., 2020). In trial with potato, a relatively large amount of microbial biomass was due to the sampling time taken in spring immediately after the planting of potato tubers, when potato production has not yet had a negative effect. On the other hand, a relatively smaller amount of microbial biomass in the experiment with spring barley may have been due to the effect of potato from the previous year. Recovery of biological properties after potato could take 3–4 years (Nelson et al., 2009). Therefore, it is essential to use more than three-year rotations when using potato production to allow the recovery of microbial properties and retain soil health after potato.

Secondly, the experiment with potato was fertilised 20 t ha<sup>-1</sup> of composted cattle manure compared to 10 t ha<sup>-1</sup> in the experiment with spring barley. Cattle manure has positive effect to soil microbiology (Edesi et al., 2020). In addition, relatively larger amount of microbial biomass in the experiment with potato could be a result of pea as the main crop in the previous year, because legumes have a positive effect on microbial activities (Dhakal, Islam, 2018). The differences between microbial biomass content in fertilisation treatments were similar when data were pooled together regardless of main crops (Figure 1, Table 3).

A comparison of different microbial groups by treatments showed that all microbial groups were higher in OrgII and OrgI treatments than in the conventional system in both experimental (potato and spring barley) fields (Figure 1, Table 3). Cover crops had positive effect to microbial biomass content and microbial community structure in soil (Rankoth et al., 2019). Inversely, conventional farming with NPK fertilisation (ConvII) and no fertiliser using only pesticide (Conv0) showed



Note. Explanation under Table 2; PLFA – phospholipid fatty acid; different letters indicate significant differences between potato (capital letters) and barley (lower-case letters) treatments (Tukey's test,  $p > 0.05$ ); error bars represent standard deviation of the mean (n = 3).

**Figure 1.** Mean values of microbial biomass of microbial groups by main crops in a long-term five-field crop rotation experiment

significantly the smallest number of total FAMES, Gram-negative, Gram-positive, total bacteria, and fungi ( $p < 0.05$ ). ConvII treatment showed reducing effect on microbial biomass content and activity (Angelini et al., 2013; Edesi et al., 2012; 2020). In the organic farming system without any external organic amendments, microbial community relies only cycling of plant material in the field; therefore, it is important to return crop residues to the soil (Arcand et al., 2016). In both experiments, ConvII and Conv0 treatments showed the lowest values compared to OrgII and OrgI ones (Figure 1, Table 3). This agrees with the results of previous studies which showed that chemical pesticides had negative effect on soil microorganisms (Edesi et al., 2012; Angelini et al., 2013). In more detail, Gram-negative bacteria are

small and sensitive to different stress conditions, e.g., drought, availability of nutrients, and low pH. In addition, Gram-negative bacteria are positively related with easily available plant-derived C sources like plant residues (Fanin et al., 2019; Moreno et al., 2021). For example, the abundance of those bacteria increased with different legumes (Panettieri et al., 2020; Moreno et al., 2021). The largest number of Gram-negative bacteria was found in OrgII treatment in potato as well as in spring barley (Figure 1, Table 3). In potato, OrgI treatment showed

similar values to control plots. In spring barley, the amount of Gram-negative in OrgI treatment was similarly as high as in OrgII one. Both Conv0 and ConvII treatments showed the smallest number of Gram-negative bacteria. The results of previous studies showed that the addition of organic amendments caused an increase in Gram-negative bacteria (Bray et al., 2012). Therefore, residues of the main crop or cover crops increase the content of Gram-negative bacteria. Thus, the use of cover crops provided an additional increase in this bacterial group.

**Table 3.** Mean values ( $\pm$  SD) ( $n = 3$ ) of phospholipid fatty acid (PLFA) ( $\text{nmol g}^{-1}$  dry soil) in a long-term five-field crop rotation experiment

Treatment	Total PLFAs	Total bacteria	Gram-positive	Gram-negative	<i>Actinobacteria</i>	Fungi
Control	50.17 ab ( $\pm$ 8.46)	26.25 ab ( $\pm$ 4.44)	10.92 ab ( $\pm$ 1.89)	12.83 ab ( $\pm$ 2.34)	3.95 ab ( $\pm$ 3.95)	1.50 a ( $\pm$ 0.51)
OrgI	56.36 a ( $\pm$ 4.92)	29.27 a ( $\pm$ 2.46)	11.87 a ( $\pm$ 1.02)	14.63 a ( $\pm$ 1.31)	4.63 a ( $\pm$ 4.63)	1.67 a ( $\pm$ 0.43)
OrgII	55.61 a ( $\pm$ 6.16)	29.04 a ( $\pm$ 3.38)	12.03 a ( $\pm$ 1.40)	14.38 a ( $\pm$ 2.04)	4.43 ab ( $\pm$ 0.43)	1.42 ab ( $\pm$ 0.33)
Conv0	40.04 b ( $\pm$ 5.90)	20.92 b ( $\pm$ 3.06)	8.42 c ( $\pm$ 1.48)	10.19 b ( $\pm$ 1.48)	3.50 c ( $\pm$ 0.28)	1.02 ab ( $\pm$ 0.45)
ConvII	40.85 b ( $\pm$ 4.63)	21.28 b ( $\pm$ 2.48)	8.84 bc ( $\pm$ 0.94)	10.18 b ( $\pm$ 1.38)	3.92 bc ( $\pm$ 0.33)	0.78 b ( $\pm$ 0.11)

Explanation under Table 2

Gram-positive bacteria are large cells with thicker cell walls. They are usually more resistant to different stress conditions, e.g., deficit of water and nutrients and low pH. This group reacted similarly to Gram-negative bacteria when comparing organic and conventional treatments. The highest values of Gram-positive bacteria were found in OrgI and OrgII treatments (Table 3). The results of various studies showed larger number of Gram-positive bacteria due to organic amendments like plant residues (Bray et al., 2012) and cover crops and manure (Abaye et al., 2005). In addition, plant-based organic fertilisers increase functional diversity of microbial community (Toleikiene et al., 2021). It is common knowledge that Gram-positive bacteria decompose more recalcitrant soil organic matter as C source. For example, a strong association with complex C forms like carbonyls has been noticed (Fanin et al., 2019). In addition, microbes utilise different forms of C sources from various organic amendments at different speed (Toleikiene et al., 2021). The use of an older C source from soil was also supported by the C:N ratio. Regardless of significantly lower percentages of SOC in conventional management system, the C:N ratio was the highest in treatments with mineral fertilisers and chemical pesticides (ConvII). In conclusion, the results of current experiment support the hypothesis that Gram-positive bacteria use the old C source from soil.

*Actinobacteria* was significantly the highest ( $p < 0.05$ ) in the organic farming system with only cover crops and no manure addition (OrgI) in spring barley plots  $4.93 \text{ nmol g}^{-1}$  dry soil and in potato plots  $4.87 \text{ nmol g}^{-1}$  dry soil (Figure 1). In OrgII treatment, the amount of *Actinobacteria* was similar to control treatments in both experiments. This could be explained with the results from previous studies, where *Actinobacteria* appeared more dominant in organic farming systems compared to conventionally managed soils, especially during later stages of decomposition (Arcand et al., 2016). Still, composted cattle manure had some negative effect to the group of *Actinobacteria* compared to the treatments with only cover crops in the organic farming system.

The results of previous studies showed that relative abundance of FAMES belonging to the group of *Actinobacteria* decreased with available phosphorus (Bünemann et al., 2004; Allison et al., 2007). In current experiment, an added amount of phosphorus with composted cattle manure could be impact of *Actinobacteria*. In addition, research with acidified and untreated pig slurry also showed a lower amount of *Actinobacteria* in all slurry treated plots compared to the treatments with mineral fertilisers (Edesi et al., 2020). Thus, solely cover crops have positive effect, and manure addition could have negative impact on *Actinobacteria*.

Fungi are important microbial group for the breakdown of the complex compounds, such as cellulose and lignin, especially at the beginning of decomposition (Xu et al., 2020). In addition, Shahbaz and colleagues (2020) found that fungi played an important role in utilisation of SOM from old as well as young C pools and that they were the most active group in the rhizosphere (Shahbaz et al., 2021).

The results of current experiment showed the highest values of fungi in treatments only with cover crops (OrgI); still, these values were slightly lower in the treatments with cover crops and manure (Figure 1). Significantly lower number of fungi in all treatments of conventional farming indicates the negative effect of chemical pesticides compared to the results of treatment with no fertilisation (control). It can be concluded that the main reason for the decrease in fungi in ConvII and Conv0 treatments could be due to used chemical pesticides, especially as fungicides.

In conclusion, soil microbial biomass content in total and by different microbial groups was higher in fertilisation treatments of organic farming systems indicating a positive effect of cover crops and manure addition. In addition, main crops and previous crops have an influence on microbial biomass content.

**Soil microbial community structure.** In spring, the relative abundance (% mol) of microbial groups did not differ significantly between treatments and farming systems except for *Actinobacteria* (Table 4).

Soil microbial community is strongly affected by season, fertilisation, and management. Seasonal effect could be the result of soil mineral-N, precipitation (Bardgett et al., 1999), and temperature (Yokobe et al., 2018). During the sampling period, weather conditions in April were warmer than the long-term average but extremely dry (4 mm precipitation per April). Hence, warmer temperature could stimulate soil microbiology, but moisture is one of the key factors in the development of soil microbial community after the dormant period. The  $N_{\text{tot}}$  before fertilisation in spring was similar in all treatments (Table 2), but soil mineral-N content was one of seasonal factors (Bardgett et al., 1999). Another reason for similar relative abundance of different microbial groups could be result of intensive soil mixing due to ploughing. The composition of microbial community is more related to tillage than crop rotation (Zhang et al., 2014). The results of current experiment are also in accordance with the results of previous studies, where long-term cattle manure application increased microbial biomass content without changing the community composition (Ma et al., 2020). In addition, bacterial abundance was similar regardless of whether they were unfertilised or fertilised with soil mineral-N in a long-term field experiment in

Sweden (Shahbaz et al., 2021). In conclusion, the results of current experiment showed that neither fertilisation nor crop rotation had a significant effect on the structure of microbial community composition in spring.

Only *Actinobacteria* showed higher values in Conv0 and ConvII treatments compared to OrgI and OrgII ones (Figure 2; Table 4). Specifically, the highest values ( $p < 0.05$ ) of *Actinobacteria* were found in ConvII treatment (Figure 2). The positive effect of mineral fertilisers on the relative abundance of *Actinobacteria* was previously shown when compared to pig slurry treatments (Edesi et al., 2020), but relatively high values were also found in treatments only with chemical pesticides and no mineral fertilisation (Conv0) (Figure 2; Table 4).

Hence, the use of chemical pesticides alone increased the relative abundance of *Actinobacteria*. *Actinobacteria* showed significant increases in the treatment with herbicide, insecticide, and fungicide indicating important involvement in the decomposition

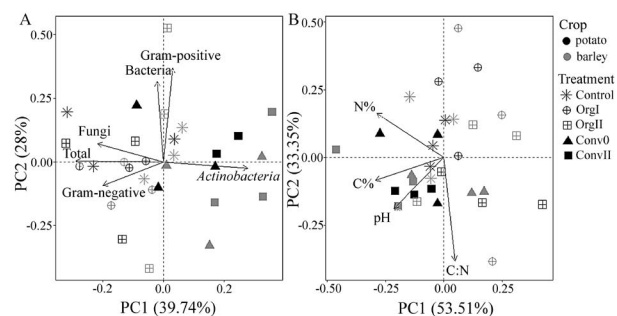
**Table 4.** Relative abundance of microbial groups (% mol) by treatments and main crops

	Treatment	Gram-positive	Gram-negative	<i>Actinobacteria</i>	Fungi
Potato	control	21.74 a	26.02 a	7.75 a	3.17 a
	OrgI	21.37 a	25.85 a	8.42 a	3.27 a
	OrgII	20.95 a	26.59 a	8.07 a	2.75 a
	Conv0	21.53 a	25.16 a	8.53 a	2.96 a
	ConvII	21.68 a	25.27 a	9.25 a	1.95 a
Spring barley	control	21.79 a	25.04 a	8.12 b	2.70 a
	OrgI	20.78 a	26.10 a	8.03 b	2.61 a
	OrgII	22.44 a	25.00 a	7.92 b	2.32 a
	Conv0	20.43 a	25.79 a	9.13 ab	2.05 a
	ConvII	21.64 a	24.50 a	10.05 a	1.98 a

Note. Explanation under Table 2; different letters indicate significant differences between treatments with same crop rotation (potato or spring barley with red clover) according to Tukey's test ( $p < 0.05$ ).

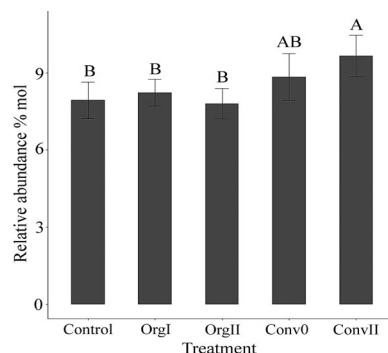
of pesticides in soil (Astaykina et al., 2020). Thus, the important conclusion is that chemical pesticides alone or in combination with mineral fertilisers increase the presence of *Actinobacteria*. To assess the effect of the conventional farming system on soil microbial community, the changes in the abundance of *Actinobacteria* could be used. Further research is essential to understand the exact mechanisms between *Actinobacteria* and the conventional farming system.

Similar results indicate a principal component analysis (PCA) of chemical properties of soil and relative abundance of microbial groups (Figure 3). Most of the variation is due to organic vs. conventional farming system. There is clear clustering according to farming systems. Conv0 and ConvII treatments are mainly located on the right side of the graph according to the relative abundance of microbial groups (A) and to soil chemical properties (B). The OrgI and OrgII treatments are placed on the left side, and the treatments with no fertilisation (control) are positioned mainly in the centre of the graph. A biplot of microbial groups indicated that soil from the organic farming system have higher relative abundance



Explanation under Table 2

**Figure 3.** A biplot of principal component (PC) analysis according to the relative abundance of phospholipid fatty acid (PLFA) (% mol) (A) and soil chemical properties (B)



Explanation under Table 2; the error bars represent standard deviation of the mean (n = 3).

**Figure 2.** Relative abundance of *Actinobacteria* in a long-term five-field crop rotation experiment by treatments

in all microbial groups except for *Actinobacteria* compared to soil of conventional farming. All samples with chemical pesticides and mineral fertilisers (ConvII) or only chemical pesticides (Conv0) are clearly placed on the right side of the graph.

These results confirm data of our previous findings with ANOVA and descriptive statistics that conventional management system has smaller amount of all microbial groups. Higher abundance is in accordance with higher values of C% and pH in OrgI and OrgII treatments (Table 2) indicating increased SOC in organic farming system due to organic amendments (cover crops and composted cattle manure). Different organic amendments, conventional tillage, and cover cropping have positive effect on SOC and microbial biomass C for a longer period of adaptation (Crystal-Ornelas et al., 2021). Thus, both chemical analyses of soil and the structure of microbial community change in the same direction, even if these changes are not so clear when looking at each parameter alone.

Consequently, to clarify the influence of crops and sampling time for microbial community, more studies are needed from different rotations and sampling points. For example, Panettieri et al. (2020) pointed out that more reliable data could be after harvest before tillage due to crop variability.

### Conclusions

1. The organic farming system, such as cover crops and cover crops with composted cattle manure, improve chemical properties of soil. Long-term five-field crop rotation experiment showed increases in soil pH and soil organic carbon (SOC) content compared to the conventional farming system using mineral fertilisers and chemical pesticides.

2. Main crops and previous crops had a direct impact on soil microbial biomass content but no significant effect on soil microbial community structure. Intensive tillage or cultivation of legumes affects microbial biomass

content, but the proportion of different microbial groups in the microbial community did not change.

3. Different fertilisation practises have an impact on soil microbial biomass content. The use of organic amendments, such as cover crops and cover crops with cattle manure as a part of organic farming system, had a positive effect on total soil microbial biomass content and, also, on microbial community structure. A higher content of Gram-negative bacteria was found in farming systems with cover crops due to extra amount of plant residues, which provide C sources for these microorganisms. On the other hand, more SOC in organic farming systems was favourable for Gram-positive bacteria.

4. The relative abundance of microbial community did not show significant differences between farming systems regarding fertilisation practices except for higher abundance of *Actinobacteria* in the conventional farming system. Thus, chemical pesticides alone or in combination with mineral fertilisers had a positive effect to the presence of *Actinobacteria*. The changes of *Actinobacteria* content in conventional farming system suggest an impact of fertilisers on conventional agriculture.

5. Soil chemical properties were in accordance with the results from phospholipid fatty acid (PLFA) analysis and thus could be used to estimate soil microbial biomass content and microbial community structure in agricultural soils.

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## Mikroorganizmų bendrijos sudėtis ilgalaikėse ekologinio ir tradicinio ūkininkavimo sistemose

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### Santrauka

Tradicinė žemdirbystė daro didelį poveikį gamtai, labiausiai dėl mineralinių trąšų ir cheminių pesticidų naudojimo. Šiandien ekologinis ūkininkavimas vis dažniau pereina prie atkuriamosios žemdirbystės. Ilgalaikio penkių laukų sėjomainos eksperimento metu buvo tirta skirtingo tręšimo įtaka ekologinio ir tradicinio ūkininkavimo sistemoms. Dirvožemio cheminės savybės, mikroorganizmų biomasės kiekis ir bendrijų struktūra tirta lauko eksperimente su žieminiiais dengiamaisiais augalais (OrgI), žieminiiais dengiamaisiais augalais su kompostuotu mėšlu (OrgII) kaip ekologinio ūkininkavimo sistema, ją lyginant su tradicine ūkininkavimo sistema su mineralinėmis trąšomis ir cheminiais pesticidais (ConvII) arba tik su cheminiais pesticidais be tręšimo (Conv0). Kontrolinis variantas buvo laukeliai be papildomų organinių priedų arba mineralinių trąšų. Vykdyti du lauko eksperimentai su skirtingais pagrindiniais augalais: bulvėmis (priešėlis žirniai) ir vasariniais miežiais su raudonųjų dobilų išėliu (priešėlis bulvės). Lyginant su tradicine ūkininkavimo sistema, visų grupių mikroorganizmų buvo daugiau abiejose (bulvių ir miežių) ekologinio ūkininkavimo sistemose (OrgII ir OrgI). Eksperimente su bulvėmis mikroorganizmų biomasės vertės buvo didesnės, tačiau tręšimo skirtumai, nepriklausomai nuo ūkininkavimo praktikos, buvo nedideli. Eksperimente su miežiais ir raudonaisiais dobilais nustatyta mažesnė mikroorganizmų biomasė, tačiau biomasė pagal mikroorganizmų grupes (gramteigiamos bei gramneigiamos bakterijos, visos bakterijos, aktinobakterijos ir grybai) buvo gerokai didesnė laukeliuose su organiniais priedais. Santykinis mikroorganizmų bendrijos gausumas tarp ūkininkavimo sistemų arba tręšimo būdų reikšmingai nesiskyrė, išskyrus didesnį kiekį aktinobakterijų tradicinėje ūkininkavimo sistemoje, rodantį reikšmingą pesticidų poveikį šiai grupei. Nustatyta, kad ekologinio ūkininkavimo sistema padeda išlaikyti arba padidinti gausą dirvožemio mikroorganizmų bendrijų, kurios yra svarbi dirvožemio sveikatingumo dalis.

Reikšminiai žodžiai: ilgalaikis lauko našumo eksperimentas, sėjomaina, dengiamieji augalai, mėšlas, mineralinės trąšos, fosfolipidinė riebalų rūgštis.