

## Article

# Soil Microbial Activity in Different Cropping Systems under Long-Term Crop Rotation

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**Abstract:** Soil microbes play a key role in the nutrient cycling by decomposing the organic material into plant-available elements and also by maintaining the soil health. The study of soil microbial hydrolytic activity (SMA) was carried out in a long-term crop rotation (barley undersown (us) with red clover, red clover, winter wheat, pea and potato) experiment in five different farming systems during 2014–2018. There were two conventional systems, with chemical plant protection and mineral fertilizers, and three organic systems, which included winter cover crops and composted manure. The aim of the present study was to evaluate the effect of the (i) cropping system and (ii) precrops in rotation on the soil SMA. The soil microbial hydrolytic activity was significantly affected by yearly weather conditions, farming system, and crops. In all farming systems, the SMA was the lowest after dry and cold conditions during early spring in 2018. In unfertilized conventional systems, the considerably lower SMA is explained by the side effects of pesticides and low organic residuals, and we can conclude that the conventional system with no added fertilizer or organic matter is not sustainable, considering soil health. In each year, the SMA of organic systems with cover crops and composted manure was 7.3–14.0% higher compared to all farming systems. On average, for both farming systems, the SMA of all the rotation crops was positively correlated with the SMA values of precrops. However, in conventional farming systems, the effect of undersowing on the SMA of the precrop was smaller compared to organic systems.

**Keywords:** conventional system; organic system; cover crops; manure; soil microbial activity

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## 1. Introduction

Biotic (microbial biomass, enzymatic activities, etc.) and abiotic parameters (pH,  $C_{org}$ ,  $N_{tot}$ , etc.) are used for the evaluation of soil health and quality. Generally, more attention is paid to the physical and chemical soil parameters, leaving the biological factors (microbial and enzymatic activities) unattended. However, biological properties are more sensitive to the changes occurring in the soil as compared to the physical and chemical parameters. Therefore, the changes in the soil microbial populations and their activities could be suitable indicators for evaluation of soil health.

Soil microorganisms make up less than 1% of total soil mass, but they play an important functional role in supporting the soil ecosystem [1]. Soil microorganisms are a significant part of soil fertility because they facilitate the decomposition of organic matter and formation of humus [2,3]. Microbes play a key role in soil nutrient cycling by decomposing the organic material and transforming it into plant-available inorganic compounds [4–6] and also by maintaining the soil health [7,8]. From the plant health

perspective, the importance of the interaction of plant roots and local microbial communities cannot be overstated. This has a positive impact on crops through growth-promoting and/or pathogen-suppressing effects; vice versa, field crops have an effect on soil microbiota in the rhizosphere, i.e., through attracting microbes and stimulating root growth [9]. Soil microorganisms also regulate the amount of carbon fixed in the soil and released back to the atmosphere, thereby indirectly regulating the productivity of organic carbon stored in the soil [10,11]. Weather conditions, soil moisture, pH, soil management, plant protection measures, fertilization, and other factors have a significant impact on the population and composition of species of microbes [12–16]. In organic production where only organic fertilizers are used, the amount and quality of fertilizers affect the soil physico-chemical properties as well as the microbial biomass and its activity in the soil [17–19]. Compared to the soil physical and chemical parameters, the activity of microbes is much more susceptible to various changes [20,21].

The objective of the present study is to evaluate the effect of the (i) cropping system and (ii) precrops in rotation on the soil microbial hydrolytic activity (SMA) during a 5-year period in an ongoing long-term five-field crop rotation experiment with different farming systems.

We hypothesized that (i) the cropping system has a significant impact on the SMA and (ii) the soil SMA depends on the precrop in the crop rotation.

## 2. Materials and Methods

### 2.1. Site Description

In 2008, a five-field crop rotation experiment was set up at the Rõhu Experimental station (Estonian University of Life Sciences, Tartu, Estonia) in Eerika (58°21' N, 26°39' E). Data for this study were collected during 2014–2018. The soil was described as Stagnic Luvisol in the World Reference Base for soil resources [22] classification, with 56.5% sand, 34% silt, and 9.5% clay, and a 27 to 29 cm depth of the ploughing layer [23]. The mean characteristics of the humus horizon were  $C_{org}$  1.1–1.2%,  $N_{tot}$  0.10–0.12%, P 110–120 mg kg<sup>-1</sup>, K 253–260 mg kg<sup>-1</sup>, pH<sub>KCl</sub> 5.9–6.1, and soil bulk density 1.45–1.50 g cm<sup>-3</sup>. Estonia lies in the northern part of the temperate climate zone and in the transition zone between maritime and continental climates. Local climatic differences are due, above all, to the neighboring Baltic Sea, which warms up the coastal zone in winter and has a cooling effect, especially in spring [24].

### 2.2. Experimental Design

Experiments were set up as systematic block design with four replications. The size of the plot was 60 m<sup>2</sup>. In the present research, the data were gathered from 5-field crop rotation during 2014–2018. Once a year in mid-April, before starting the field operations, soil samples were taken from a depth of 0–25 cm. Eight samples were taken from each plot to obtain the average for the plot. Crops in the rotation were as follows: barley cultivar 'Anni', which was undersown by red clover; red clover tetraploidy cultivar 'Varte'; winter wheat cultivar 'Fredis'; pea cultivar 'Starter'; and potato cultivar 'Maret'. Cereal straw and red clover biomass were ploughed into the soil.

### 2.3. Fertilizer and Crop Management

The experiment had three organic (ORG) and two conventional (CON) systems. (Table 1). In the CON 0 treatment in the conventional farming system, no fertilizers were used; only chemical plant protection products were applied. In the CON 1 treatment plots of undersown barley, potato, and winter wheat phosphorus, (P) 25 kg ha<sup>-1</sup> and potassium (K) 95 kg ha<sup>-1</sup> were applied. In addition, nitrogen fertilizer was applied: 120 kg N ha<sup>-1</sup> for undersown barley, 150 kg N ha<sup>-1</sup> for potato and winter wheat, and 20 kg N ha<sup>-1</sup> for pea. The red clover plots were not fertilized in conventional farming systems. After the harvest of potato, pea, and winter wheat, glyphosate containing herbicide (3.0 l ha<sup>-1</sup>) was applied for weed management.

During the growing season, weed management MCPA-750 (active ingredient MCPA-750 g ha<sup>-1</sup>) with the rate of 1.0 l ha<sup>-1</sup> for barley undersown with red clover and 0.7 L ha<sup>-1</sup> for pea was used. In winter wheat, Secator OD (150 mL ha<sup>-1</sup>; active ingredients amidosulfuron 15 g ha<sup>-1</sup>, methyl-iodosulfuron sodium 3.75 g ha<sup>-1</sup>, and mefenpyr-diethyl 37.5 g ha<sup>-1</sup>), and for potato, Titus 25 DF (50 g ha<sup>-1</sup>; active ingredient rimsulfuron 12.5 g ha<sup>-1</sup>) was applied, and potato fungicide Ridomil Gold MZ 68 WG (2.5 kg ha<sup>-1</sup>; metalaxyl-M 100 g ha<sup>-1</sup> and mancozeb 1600 g ha<sup>-1</sup>) was used for late blight control. Depending on the rate of infestation, spraying was performed 2–4 times during the summer. In the conventional farming systems, potato insecticide Fastac 50 (0.3 l ha<sup>-1</sup>; alpha-cypermethrin 100 g L<sup>-1</sup>) and Decis Mega 50EW (0.15 L ha<sup>-1</sup>; active ingredient deltamethrin 7.5 g ha<sup>-1</sup>) were used against Colorado beetle.

There were 3 treatments in the organic farming system: ORG 0—without winter cover crops; ORG CC—winter cover crops were used (the mixture of turnip rape and winter rye after the main crop of winter wheat, winter turnip rape after the main crop of pea, and winter rye after the main crop of potato); ORG CC+M—in addition to cover crops mentioned previously, composted cattle manure (10 t ha<sup>-1</sup> for cereals and 20 t ha<sup>-1</sup> for potato) was ploughed into the soil in spring (Table 1). Winter cover crops were sown immediately after the harvesting of the main crop in August.

**Table 1.** The treatments in the organic and conventional farming systems Matsen et al. [25].

Crop Rotation	Organic Systems		Conventional Systems		
	ORG 0—control (Crop rotation)	ORG CC (Crop rotation + green manure as winter cover crops)	ORG CC + M (Crop rotation + green manure + composted cattle manure)	CON 0—control (Crop rotation + herbicides + fungicides + insecticides)	CON 1 (Crop rotation + herbicides + fungicides + insecticides + mineral fertilizers)
Winter wheat		Winter oilseed turnip + winter rye	10 t ha <sup>-1</sup>		25 kg ha <sup>-1</sup> P * and 95 kg ha <sup>-1</sup> K **, 150 kg ha <sup>-1</sup> N ***
Pea		Winter oilseed turnip			25 kg ha <sup>-1</sup> P and 95 kg ha <sup>-1</sup> K; 20 kg ha <sup>-1</sup> N
Potato		Winter rye	20 t ha <sup>-1</sup>		25 kg ha <sup>-1</sup> P and 95 kg ha <sup>-1</sup> K; 150 kg ha <sup>-1</sup> N
Barley undersownwith red clover			10 t ha <sup>-1</sup>		25 kg ha <sup>-1</sup> P and 95 kg ha <sup>-1</sup> K; 120 kg ha <sup>-1</sup> N
Red clover		Red clover			

Note. \*—phosphorus; \*\*—potassium, \*\*\*—nitrogen.

#### 2.4. Chemical Analysis

The spectrophotometric determination of soil microbial hydrolytic activity is a simple and fast method for evaluation of the microbial activity in the soil [26]. According to ISO 10381-6:1993 [27] for determination of soil microbial hydrolytic activity, the samples (each about 500 g fresh weight) were placed in polyethylene bags (not closed) and stored at 4 °C until analysis. Each sample was mixed individually, and larger parts of plant material and stones were picked out by hand. About 200 g (fresh weight) was taken for a sub-sample and sieved through a Ø 2 mm sieve. For the determination of the dry matter content, 5 g of homogenized soil sample was weighed in pre-weighed 50 mL beakers and dried to a constant weight (minimum 3 h) at 105 °C. After cooling to room temper-

ature in a desiccator, the samples were weighed again. The preparation of reagents following soil microbial hydrolytic activity analysis was performed according to the method described by Adam and Duncan [28]. We used acetone instead chloroform/methanol as the reaction termination reagent and optimized the incubation regime.

### 2.5. Meteorological Data

The experimental field is 59 m above sea level and belongs to the South-Estonian upland agro-climatic region, where the average annual sum of active air temperatures (sum >5 °C) is 1750–1800 °C, the mean annual temperature is +5.5 °C, and the amount of precipitation 550–650 mm. The weather during the experimental period was monitored with a Metos Model MCR300 electronic weather station (Pessl Instruments, GmbH, Weiz, Austria), which automatically calculates the average daily temperatures and the sum of precipitation. The measurements were made from the Eerika weather station at the Institute of Agricultural and Environmental Sciences of the Estonian University of Life Sciences. Compared to the long-term (1964–2018) average temperature, the mean temperatures during the experiment were higher by 0.8–1.7 °C (Table 2). A clear trend of climate warming is observable from this comparison [24]. Winter periods are milder compared to the long-term average, especially in February, which in 2014–2016 was 5.5–6 °C warmer. On the other hand, February and March 2018 were much colder than the previous study period and the long-term average; this might affect SMA results compared to other samples, because samples are taken in mid-April, and microbiological activities might be postponed because of cold soil. Precipitation fluctuated significantly, but the averages of the years are comparable. The year 2017 had the highest precipitation (55.8 mm above long-term average), which was caused by a very wet autumn period; 2015 was the driest year during the study period (Table 3).

**Table 2.** Mean temperature (°C) in 2014–2018 compared to the long-term average (1964–2018) data \*.

Month	Mean Temperature (°C)					
	2014	2015	2016	2017	2018	1964–2018
January	−7.9	−1.8	−9.4	−3.5	−2.4	−5.7
February	−0.2	−0.9	0.3	−2.9	−8.3	−5.7
March	2.2	2.7	−0.1	1.4	−3.5	−1.5
April	6.5	5.4	6.1	3.4	7.2	4.8
May	11.9	10.3	14.0	10.3	x	11.4
June	13.4	14.2	15.9	14.0	x	15.4
July	19.3	15.7	17.8	15.9	x	17.4
August	16.8	17.0	16.1	16.8	x	16.1
September	12.1	12.6	12.3	12.2	x	11.1
October	5.3	4.6	4.1	5.4	x	5.7
November	1.4	3.6	−1.0	2.4	x	0.5
December	−1.5	2.5	−0.4	0.2	x	−3.1
Average	6.6	7.2	6.3	6.3	−7.0 <sup>1</sup>	5.5

Note. \*—data from Eerika weather station, Estonia. x—out of the test period. <sup>1</sup> Average of January–April.

**Table 3.** Sum of precipitation (mm) in 2014–2018 compared to the long-term average (1964–2018) data \*.

Month	Sum of Precipitation (mm)					
	2014	2015	2016	2017	2018	1964–2018
January	25.0	29.6	34.0	27.4	20.4	28.6
February	12.4	8.4	55.8	22.4	11.7	22.6
March	9.0	12.0	23.3	17.0	12.9	22.5

April	13.4	69.0	51.6	51.5	28.1	31.2
May	83.8	62.0	1.6	15.5	x	52.9
June	103.4	39.4	124.6	94.3	x	70.9
July	71.4	61.4	81.6	60.7	x	69.2
August	113.0	41.2	42.0	106.2	x	81.1
September	22.2	59.0	15.4	83.4	x	59.3
October	35.8	10.8	33.2	75.3	x	56.3
November	10.4	53.8	45.5	26.1	x	44.7
December	41.6	46.3	30.6	51.8	x	36.6
Average	541.4	492.9	539.2	631.5	73.1 <sup>1</sup>	575.7

Note. \*—data from Eerika weather station, Estonia. x—out of the test period. <sup>1</sup> Sum of January–April.

### 2.6. Statistical Analysis

The statistical analysis of the collected data was performed with the software Statistica 13 (Quest Software Inc, Aliso Viejo, CA, USA). Full-factorial analysis of variance (ANOVA) was used to test the statistical significance of year, farming system, and crop and their interaction effects on the soil microbial hydrolytic activity, average air temperatures, and sum of precipitation. The Fisher (LSD) test [29] was applied for pairwise comparisons of the factors. Correlation analysis was used for linear correlation coefficients between variables, and the significance of coefficients was taken as  $p < 0.001$ ,  $p < 0.01$ ,  $p < 0.05$ , or ns (not significant).

## 3. Results and Discussion

The soil microbial hydrolytic activity was significantly affected by yearly weather conditions, farming system, crop, and the combined effect of farming system and crop grown (Table 4). The combined effect of year and farming system as well as year and crop were less significant, and there was no combined effect of all three factors (year, farming system, and crop).

**Table 4.** Analysis of variance for soil microbial hydrolytic activity ( $\mu\text{g}$  fluorescein  $\text{g}^{-1}$  soil dry weight  $\text{h}^{-1}$ ) depending on year, farming system, crop, and their interaction.

Source of Variation	df	SS	MS	F	p
Year (Y)	4	916	229	10.25	$p < 0.001$
Farming system (FS)	4	8669	2167	97.06	$p < 0.001$
Crop (C)	4	1607	402	17.99	$p < 0.001$
Y $\times$ FS	16	743	46	2.08	$p = 0.09$
Y $\times$ C	16	874	55	2.45	$p = 0.02$
FS $\times$ C	16	1049	66	2.94	$p < 0.001$
Y $\times$ FS $\times$ C	64	1272	20	0.89	$p = 0.710$

Notes: df—degrees of freedom; SS—sums of squares; MS—mean squares; F—treatment mean square/error mean square; p—significance probability value.

### 3.1. Changes in Soil Microbial Hydrolytic Activity during the Study Period

In unfertilized systems, the soil SMA of ORG 0 was higher by 12.8% than in CON 0 (Table 5). This shows a low organic content in CON 0, where organic matter yields are lower than in fertilized systems [30], and the negative effects of chemical pesticides on the soil microorganisms. To avoid the negative effects on soil health, Esmaeilzadeh-Salestani et al. [31] found that conventional cropping with a small to average amount of mineral fertilizer seems to eliminate the negative effect of pesticides; in addition, long and diverse crop rotations are important for microbial functional stabilization.

**Table 5.** The hydrolytic activity of soil microorganisms ( $\mu\text{g}$  fluorescein  $\text{g}^{-1}$  soil dry weight  $\text{h}^{-1}$ ) during 2014–2018.

Farming System	2014	2015	2016	2017	2018	F Stat and p Value
CON 0	48.1 <sup>A1ab2</sup>	46.7 <sup>Aab</sup>	49.8 <sup>Ab</sup>	47.4 <sup>Aab</sup>	45.5 <sup>Aa</sup>	$F_{4,95} = 1.44; p = 0.23$
CON 1	52.6 <sup>Bab</sup>	54.9 <sup>Bb</sup>	53.1 <sup>ABab</sup>	51.0 <sup>Ba</sup>	49.8 <sup>Ba</sup>	$F_{4,95} = 2.75; p = 0.032$
ORG 0	53.9 <sup>Bb</sup>	54.2 <sup>Bb</sup>	56.1 <sup>BCbc</sup>	57.6 <sup>Cc</sup>	50.8 <sup>Ba</sup>	$F_{4,95} = 7.57; p < 0.001$
ORG CC	55.3 <sup>Ba</sup>	58.6 <sup>Cb</sup>	57.2 <sup>Cab</sup>	57.9 <sup>Cab</sup>	55.4 <sup>Cab</sup>	$F_{4,95} = 1.59; p = 0.18$
ORG CC+M	58.4 <sup>Ca</sup>	59.3 <sup>Ca</sup>	63.9 <sup>Db</sup>	58.5 <sup>Ca</sup>	58.2 <sup>Ca</sup>	$F_{4,95} = 3.70; p = 0.008$
F stat and p value	$F_{4,95} = 12.77; p < 0.001$	$F_{4,95} = 20.638; p < 0.001$	$F_{4,95} = 13.398; p < 0.001$	$F_{4,95} = 19.266; p < 0.001$	$F_{4,95} = 20.70; p < 0.001$	

<sup>1</sup> Means followed by different capital letters within each column indicate significant influence of farming system (Fisher LSD test,  $p < 0.05$ ). <sup>2</sup> Means followed by different lower-case letters within each row indicate significant influence of year (Fisher LSD test,  $p < 0.05$ ). CON 0—control (Crop rotation + herbicides + fungicides + insecticides); CON 1 (Crop rotation + herbicides + fungicides + insecticides + mineral fertilizers); ORG 0—control (Crop rotation); ORG CC (Crop rotation + green manure as winter cover crop); ORG CC + M (Crop rotation + green manure + composted cattle manure).

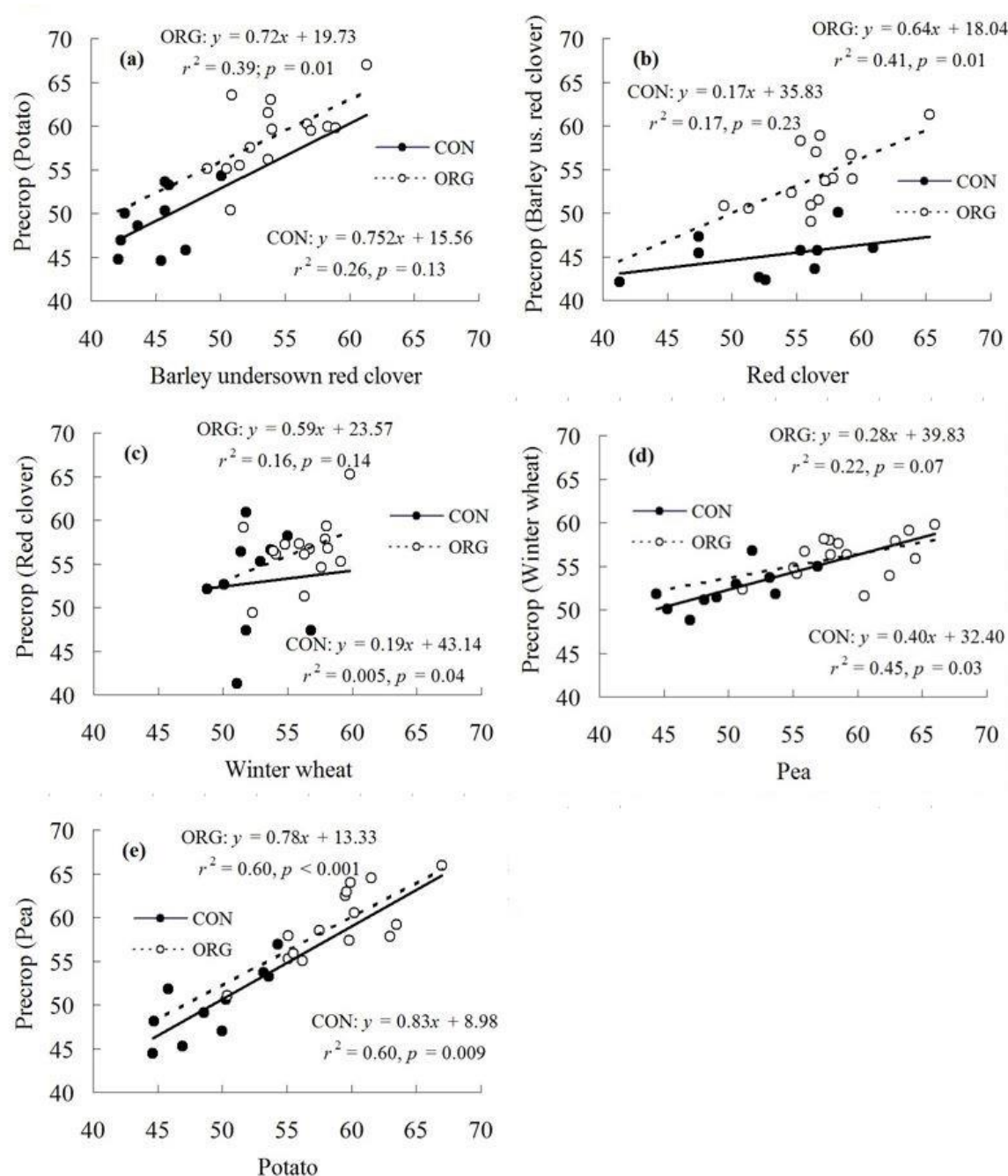
The soil SMA of ORG CC + M (manure as fertilizer) was higher by 12.7% than in CON 1 (mineral fertilizers). The soil SMA of ORG CC was higher by 2.1–7.1% than the average SMA values. The SMA value of ORG CC+M (with composted manure) was higher by 7.3–14.0% than the average SMA of all cultivation systems. Earlier studies have shown that the microbial biomass is higher in soils with high nutrient (high concentration of N, P, and K) and carbon content as well as suitable pH range for crops [32]. Cover crops and manure have a positive effect on the soil microbial activity and their abundance [33,34].

The soil moisture content is dependent on the amount of precipitation and temperature. The effect of precipitation, soil temperature, and moisture may apply directly to the activity of microbes or indirectly through factors like the amount of plant residues in the soil. The lowest soil SMA values were measured in 2018, when the soil samples were collected after a long dry and cold period (Table 3). It seems that organically managed soils are more resilient to drought and cold. Soils with higher organic matter and cover crops do not freeze to as great a depth in cold periods, and at the beginning of the season, they warm up faster and thus microbiological activities start sooner. This is related to the higher C content of organically managed soils [31,35], which also improves the water-holding capacity of these soils.

### 3.2. The Effect of Precrops on the SMA

The abundance, composition, and soil microbial hydrolytic activity are influenced by soil biochemical conditions during the growing season as well as by the precrops in rotation. Therefore, it is important to know the extent the SMA of crops is affected by the content of organic matter left in the soil or the organic matter that decomposes in the soil during the growing season.

Our study results indicated that the SMA of all the crops in the rotation of both farming systems was positively correlated with the SMA values of the precrops (Figure 1A–F).



**Figure 1.** The regression analysis of the soil hydrolytic activity ( $\mu\text{g}$  fluorescein  $\text{g}^{-1}$  soil dry weight  $\text{h}^{-1}$ ) of main crop and precrop in conventional and organic farming systems. (a) Barley undersown with red clover (precrop was potato); (b) red clover (precrop was barley undersown with red clover); (c) winter wheat (precrop was red clover); (d) pea (precrop was winter wheat); (e) potato (precrop was pea).

The relationships between the SMA values of barley undersown with clover and soil of precrop potato were almost identical in their linearly ascending characteristics (Figure 1A) and were clearly present in both farming systems. The SMA of barley undersown with red clover depended on the precrop in the conventional as well as in the organic farming systems, with respective correlations of  $r = 0.51$ ;  $p = 0.13$  and  $r = 0.63$ ;  $p = 0.01$  (Figure 1A). The higher correlative relationship in the ORG system is probably due to the fact that after the harvest of the potato precrop—pea—winter rye and winter turnip rape were sown as winter cover crops, which after being ploughed into the soil, resulted in higher organic matter content in the plots of ORG CC and ORG CC+M and hence the higher SMA values. The addition of organic matter to the soil stimulates the

growth of bacteria and actinomycetes but hinders the development of fungi [36]. By comparing the SMA values of red clover and previous barley (undersown with red clover) plots, a significant effect of the precrop barley on the SMA of red clover was seen (Figure 1B). The SMA value was probably influenced by the amount of organic matter taken into the soil by the red clover biomass. Some previous studies have also shown that the clover species undersown during the previous year, which develop into full-grown crops the next growing season, have a positive impact on the soil physical and chemical parameters [37,38]. Carter and Kunelius [39] found that the barley undersown with clover increased the root biomass by 6–11 times compared to only barley crop and also improved the structural soil characteristics. According to Skudienė and Tomchuk [40], the biomass of roots increased by up to 6.5 times and above-ground biomass by 4 times during the following season clover crop.

Our results indicated that in conventional farming systems, the effect of undersowing on the SMA of precrops was smaller ( $r = 0.41$ ;  $p = 0.23$ ) compared to organic systems ( $r = 0.64$ ;  $p = 0.01$ ). Thus, we should take into consideration that in organic farming systems, after the harvest of potato and before the barley (us), winter rye was sown as a cover crop. It was ploughed into the soil as green manure in the spring, and in the ORG CC+M treatment, manure was also applied. The higher content of organic matter received from cover crops and manure also increased the activity of microbes on the red clover plots, which resulted in stronger correlation between the SMA value of red clover and the precrop in organic systems.

After the ploughing of the clover biomass as green manure, the SMA value of the following winter wheat crop depended on the activity of decomposing soil organisms during the vegetation period. In conventional farming systems, the relationship between the SMA value of winter wheat and the precrop was insignificant ( $r = 0.07$ ;  $p = 0.04$ ). In organic systems, the same correlation was also weak ( $r = 0.40$ ;  $p = 0.14$ ). It can be concluded that the SMA value of winter wheat plots was formed mainly due to the decomposition of organic matter during the vegetation period (Figure 1C). In winter wheat plots, the correlation of SMA values with the precrop SMA of organic and conventional farming systems was in a narrow range of 48.8–59.8. This was due to the positive effect of red clover on the soil microorganisms in the CON systems, which resulted in higher SMA values, similar to ORG system. In addition to the activity of organotrophic microorganisms, the atmospheric nitrogen-fixing bacteria (*Rhizobium trifolii* and *Rhizobium leguminosarum*) in red clover and pea plants have an indirect effect on the SMA values [41].

The correlation between the SMA value of the pea plot and its precrop winter wheat was stronger in conventional farming systems compared to organic treatments (Figure 1D). In conventional farming systems,  $r = 0.67$ ;  $p = 0.03$ , and in the ORG systems,  $r = 0.47$ ;  $p = 0.07$ . This was because in the CON system plots, the SMA values depended mainly on the wheat straw ploughed into the soil; however, in the ORG treatment areas, in addition to the straw, the decomposition of organic matter from cover crops also took place, which decreased the effect of the precrop on the SMA. The residues of straw are some of the most essential organic matter deposits in the soil. Tisdale et al. [42] found that the returning of the straw residues significantly affects the functional diversity of soil microbes and enhances the activity of hydrolytical enzymes. Turk and Mihelič [43] stated that 44% of the wheat straw returned to the soil decomposed in 2 months, but full decomposition takes about 4 months.

The correlations between the SMA values of potato plots and the precrop pea were similarly strong in conventional as well as in organic farming systems:  $r = 0.77$ ; CON  $p = 0.009$  and ORG  $p < 0.001$  (Figure 1E). Compared to other crops, these correlations were the strongest, which illustrates the importance of pea as a precrop for potato, based on the SMA values. Additionally, the results of Qin et al. [44] indicate that the implementation of potato–legume crop rotation may improve the biological environment of the soil. The content of anaerobic nitrogen-fixing bacteria increased significantly in potato mon-



oculture after consecutive years, but after rotation with legumes, the abundance of aerobic azotobacteria exceeded the anaerobic competitors. At the same time, the occurrence of pathogenic fungi did not increase compared to the bacteria.

#### 4. Conclusions

Conducted field trials proved the hypothesis that cropping systems have a significant impact. Field trials showed that organic farming systems improve soil microbial activity compared to conventional systems. Organic systems with cover crops and manure significantly promote soil microbes; in all 5 years, this treatment had statistically higher activity than conventional systems. The SMA of ORG CC + M soil was 7.3–14.0% higher in all years compared to the average of the farming systems. The ORG 0 system was higher by 12.8% than CON 0; the reason for this is the limited organic matter in the CON 0 system and also the inhibiting impact of pesticides used in the CON 0 systems. Other conventional systems received additional fertilizers, which improved plant growth, and the residuals from root and shoot systems fed soil microorganisms. Adding cover crops and manure into farming systems increase the residuals and organic matter, which are used by microorganisms. From this, we can conclude that conventional systems using pesticides but no fertilizer are not sustainable. From the perspective of soil health, the best method is using cover crops and organic fertilizer. Cover crops also avoid nutrient leaching, and decomposing organic matter improves the aeration of soil, which is essential for healthy soil.

The second hypothesis, that precrops have an influence on the soil SMA, was also proven. The correlations between the SMA values of potato plots and the precrop pea were similarly strong in conventional as well as in organic farming systems,  $r = 0.77$ . Compared to other crops, these correlations were the strongest, which illustrates the importance of pea as a precrop for potato, based on the SMA values. It is also important to note that potato is an intensively cultivated crop, and this helps microorganisms to decompose organic matter. Intensive cultivation in potato growing needs cover crops, additional manure, or a well-balanced crop rotation to avoid extensive mineralization. Pea and other legume crops are suitable for sustainable crop management. Potato as a monoculture and intensive farming without cover crops should be avoided.

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#### References

1. Costa, J.; Oliveira, R.S.; Tiago, I.; Ma, Y.; Galhano, C.; Freitas, H.; Castro, P. Soil microorganisms. In *Advances in Plant Ecophysiology Techniques*; Sánchez-Moreiras, A., Reigosa, M., Eds.; Springer: Cham, Switzerland, 2018; pp. 457–482.
2. Degens, B.P.; Schipper, L.A.; Sparling, G.P.; Duncan, L.C. Is the microbial community in a soil with reduced catabolic diversity less resistant to stress or disturbance? *Soil Biol. Biochem.* **2000**, *33*, 1143–1153.
3. Kara, O.; Bolat, I. The effect of different land uses on soil microbial biomass carbon and nitrogen in Bartın Province. *Turk. J. Agric. For.* **2008**, *32*, 281–288.
4. Kennedy, A.C.; Gewin, V.L. Soil microbial diversity: Present and future considerations. *Soil Sci.* **1997**, *162*, 607–617.

5. Power, J.F.; Prasad, R. *Soil Fertility Management for Sustainable Agriculture*; CRC Press LLC: Boca Raton, NY, USA, 1997; 384p.
6. Stark, C.; Condon, L.M.; Stewart, A.; Di, H.J.; O'Callaghan, M. Influence of organic and mineral amendments on soil microbial properties and processes. *Appl. Soil Ecol.* **2007**, *35*, 79–93.
7. Arias, M.E.; Gonzalez-Perez, J.A.; Gonzalez-Vila, F.J.; Ball, A.S. Soil health—a new challenge for microbiologists and chemists. *Int. Microbiol.* **2005**, *8*, 13–21.
8. Janvier, C.; Villeneuve, F.; Alabouvette, C.; Edel-Hermann, V.; Mateille, T.; Steinberg, C. Soil health through soil disease suppression: Which strategy from descriptors to indicators? *Soil Biol. Biochem.* **2007**, *39*, 1–23.
9. Inceoglu, Ö.; van Overbeek, L.S.; Salles, J.F.; van Elsas, J.D. Normal Operating Range of Bacterial Communities in Soil Used for Potato Cropping. *Appl. Environ. Microbiol.* **2013**, *79*, 1160–1170.
10. Singh, B.K.; Bardgett, R.D.; Smith, P.; Reay, D.S. Microorganisms and climate change: Terrestrial feedbacks and mitigation options. *Nat. Rev. Microbiol.* **2010**, *8*, 779–790.
11. Bardgett, R.; van der Putten, W. Belowground biodiversity and ecosystem functioning. *Nature* **2014**, *515*, 505–511.
12. Costa, A.L.; Paixao, S.M.; Cacador, I.; Carolino, M. CLPP and EEA profiles of microbial communities in salt marsh sediments. *J. Soils Sediments* **2007**, *7*, 418–425.
13. Piton, G.; Legay, N.; Arnoldi, C.; Lavorel, S.; Clement, J.C.; Foulquier, A. Using proxies of microbial community-weighted means traits to explain the cascading effect of management intensity, soil and plant traits on ecosystem resilience in mountain grasslands. *J. Ecol.* **2020**, *108*, 876–893.
14. Zhang, Y.T.; Du, R.; Chen, H.L.; Du, P.R.; Zhang, S.J.; Ren, W.S. Different characteristics of microbial diversity and special functional microbes in rainwater and topsoil before and after 2019 new coronavirus epidemic in Inner Mongolia Grassland. *Sci. Total Environ.* **2022**, *809*, 151088.
15. Kuht, J.; Eremeev, V.; Talgre, L.; Alaru, M.; Loit, E.; Mäeorg, E.; Esmaeilzadeh-Salestani, K.; Luik, A. Changes in the soil microbial hydrolytic activity and the content of organic carbon and total nitrogen by growing spring barley undersown with red clover in different farming systems. *Agriculture* **2019**, *9*, 146.
16. Eremeev, V.; Talgre, L.; Kuht, J.; Mäeorg, E.; Esmaeilzadeh-Salestani, K.; Alaru, M.; Loit, E.; Runno-Paurson, E.; Luik, A. The soil microbial hydrolytic activity, content of nitrogen and organic carbon were enhanced by organic farming management using cover crops and composts in potato cultivation. *Acta Agric. Scand. Sect. B Soil Plant Sci.* **2020**, *70*, 87–94.
17. Gomez, E.; Ferreras, L.; Toresani, S. Soil bacterial functional diversity as influenced by organic amendment application. *Biore-sour. Technol.* **2006**, *97*, 1484–1489.
18. Gomiero, T.; Pimentel, D.; Paoletti, M.G. Environmental impact of different agricultural management practices: Conventional versus organic agriculture. *Crit. Rev. Plant Sci.* **2011**, *30*, 95–124.
19. Bonilla, N.; Cazorla, F.M.; Martínez-Alonso, M.; Hermoso, J.M.; González-Fernández, J.; Gaju, N.; Landa, B.B.; de Vicente, A. Organic amendments and land management affect bacterial community composition, diversity, and biomass in avocado crop soils. *Plant Soil.* **2012**, *357*, 215–226.
20. Oldare, M.; Pell, M.; Svensson, K. Changes in soil chemical and microbiological properties, during 4 years of application of various organic residues. *Waste Manag.* **2008**, *28*, 1246–1253.
21. Oldare, M.; Arthurson, V.; Pell, M.; Svensson, K.; Nehrenheim, E.; Abubaker, J. Land application of organic waste—Effects on the soil ecosystem. *Appl. Energy* **2011**, *88*, 2210–2218.
22. Deckers, J.A.; Nachtergale, F.O.; Spaargarn, O.C. *World Reference Base for Soil Resources: Introduction*, 1st ed.; Acco: Leuven, Belgium, 1998; p. 165.
23. Reintam, E.; Köster, T. The role of chemical indicators to correlate some Estonian soils with WRB and soil taxonomy criteria. *Geoderma* **2006**, *136*, 199–209.
24. Ministry of the Environment, Estonia's Fifth National Report under the UN Framework Convention on Climate Change. 2009. Available online: [https://unfccc.int/sites/default/files/resource/est\\_nc5.pdf](https://unfccc.int/sites/default/files/resource/est_nc5.pdf) (accessed on 15 April 2020).
25. Madsen, H.; Talgre, L.; Eremeev, V.; Sanches De Cima, D.; Luik, A. The effect of farming system on soil microbial hydrolytical activity; Programme and Abstracts: Long-term Agroecosystem Sustainability: Links between Carbon Sequestration in Soils, Food Security and Climate Change. In Proceedings of the International Scientific Conference AgroEco2016, Kaunas, Lithuania, 4–6 October 2016; pp. 42–45.
26. Schnürer, J.; Rosswall, T. Fluorescein Diacetate Hydrolysis as a Measure of Total Microbial Activity in Soil and Litter. *Appl. Environ. Microbiol.* **1982**, *43*, 1256–1261.
27. ISO 10381-6; Soil Quality—Sampling. Guidance on the Collection, Handling and Storage of Soil for the Assessment of Aerobic Microbial Processes in Laboratory. International Organization for Standardization: Geneva, Switzerland, 1993.
28. Adam, G.; Duncan, H. Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. *Soil Biol. Biochem.* **2001**, *33*, 943–951.
29. Statsoft. *Statistica*, version 7.0; Copyright 1984–2005; StatSoft Inc.: Tulsa, OK, USA, 2005; 716p.
30. Keres, I.; Alaru, M.; Eremeev, V.; Talgre, L.; Luik, A.; Loit, E. Long-term effect of farming systems on the yield of crop rotation and soil nutrient content. *Agric. Food Sci.* **2020**, *29*, 210–221.
31. Esmaeilzadeh-Salestani, K.; Bahram, M.; Ghanbari, M.; Seraj, R.; Gohar, D.; Tohidfar, M.; Eremeev, V.; Talgre, L.; Khalegh-doust, B.; Mirmajlessi, S.M.; et al. Cropping systems with higher organic carbon promote soil microbial diversity. *Agric. Ecosyst. Environ.* **2021**, *319*, 107521.

32. Aosaar, J.; Varik, M.; Lõhmus, K.; Ostonen, I.; Becker, H.; Uri, V. Long-term study of above- and below-ground biomass production in relation to nitrogen and carbon accumulation dynamics in a grey alder (*Alnus incana* (L.) Moench) plantation on former agricultural land. *Eur. J. For. Res.* **2013**, *132*, 737–749.
33. Matt, D.; Eremeev, V.; Tein, B.; Roasto, M.; Pehme, S.; Luik, A. The metabolomic fingerprinting and microbiological quality of winter wheat (*Triticum aestivum* L.) in different organic growing systems. In Proceedings of the 4th ISOFAR Scientific Conference “Building Organic Bridges”: Organic World Congress 2014, Istanbul, Turkey, 13–15 October 2014; Rahmann, G., Aksoy, U., Eds.; ISOFAR: Istanbul, Turkey, 2014; pp. 227–229.
34. Sánchez de Cima, D.; Tein, B.; Eremeev, V.; Luik, A.; Kauer, K.; Reintam, E.; Kahu, G. Winter cover crop effects on soil structural stability and microbiological activity in organic farming. *Biol. Agric. Hort.* **2016**, *32*, 170–181.
35. Kauer, K.; Pärmpuu, S.; Talgre, L.; Eremeev, V.; Luik, A. Soil Particulate and Mineral-Associated Organic Matter Increases in Organic Farming under Cover Cropping and Manure Addition. *Agriculture* **2021**, *11*, 903.
36. Kanazawa, S.; Asakawa, S.; Takai, Y. Effect of fertilizer and manure application on microbial numbers, biomass, and enzyme activities in volcanic ash soils. I. Microbial numbers and biomass carbon. *Soil Sci. Plant Nutr.* **1988**, *34*, 429–439.
37. Russell, E.W. Soil structure: Its maintenance and improvement. *Eur. J. Soil Sci.* **1971**, *22*, 137–150.
38. Christensen, B.T. Matching measurable soil organic matter fractions with conceptual pools in simulation models of carbon turnover: Revision of model structure. In *Evaluation of Soil Organic Matter Models*; Powlson, D.S., Smith, P., Smith, J.U., Eds.; Springer: Berlin/Heidelberg, Germany, 1996; pp. 143–159.
39. Carter, M.R.; Kunelius, H.T. Effect of undersowing barley with annual ryegrasses or red clover on soil structure in a barley-soybean rotation. *Agric. Ecosyst. Environ.* **1993**, *43*, 245–254.
40. Skudiene, R.; Tomchuk, D. Root mass and root to shoot ratio of different perennial forage plants under western Lithuania climatic conditions. *Rom. Agric. Res.* **2015**, *35*, 209–219.
41. Fenchel, T.; King, G.M.; Blackburn, H. Chapter 9—Symbiotic Systems. In *Bacterial Biogeochemistry: The Ecophysiology of Mineral Cycling*, 3rd ed.; Academic Press: Amsterdam, The Netherlands, 2012; pp. 163–181.
42. Tisdale, S.L.; Nelson, W.L.; Beaton, J.D. *Soil Fertility and Fertilizers*, 5th ed.; Macmillan Publishing Company: New York, NY, USA, 1985; p. 634.
43. Turk, A.; Mihelič, R. Wheat straw decomposition, N-mineralization and microbial biomass after 5 years of conservation tillage in Gleysol field. *Acta Agric. Slov.* **2013**, *101*, 69–75.
44. Qin, S.; Yeboah, S.; Cao, L.; Zhang, J.; Shi, S.; Liu, Y. Breaking continuous potato cropping with legumes improves soil microbial communities, enzyme activities and tuber yield. *PLoS ONE* **2017**, *12*, e0175934.