Article

Effects of Cover Crop and Tillage Method Combinations on the Microbiological Traits of Spring Wheat (*Triticum aestivum* L.)

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Abstract: We carried out multivariate characterisation of phenotypic variability in 27 treatments that were combinations of cover crop, tillage method, and year. Canonical variate analysis was employed to observe nine microbiological traits in an experiment established in a split-plot design. Between 2011–2013, a field experiment was conducted on soils classified as *Albic Luvisols*. The first-order factor was analysis of the effect of sowing a stubble cover crop: (Control: No cover crop sown; cover crop sown after skimming; no tillage: Cover crop sown directly). The second experimental factor involved evaluating the effects of three tillage methods (direct sowing; simplified tillage to a depth of 12–15 cm with a combined cultivator; spring ploughing to a depth of 25 cm) on nine microbiological traits. The year of research was used as a factor to differentiate between the count and activity of soil microorganisms. The traits (moulds and copiotrophic bacteria, and oligotrophic bacteria and actinobacteria) were significantly correlated (positively or negatively) at all five terms. Pearson's test confirmed the relationships between the physiological groups of soil microorganisms after the application of organic matter, and captured the positive correlation between moulds and copiotrophs at all terms of the study.

Keywords: canonical variate analysis; microorganism; spring wheat; white mustard; mahalanobis distance; quantitative traits

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1. Introduction

Long-term conventional agricultural studies provide vital information on low biomass cropping systems, where the integration of reduced tillage and cover cropping could help compensate for low carbon (C) inputs to the soil. The effect of management practices, such as reduced tillage, cover cropping, and nitrogen fertilisation on soil quality indicators is dependent on time [1]. Measurement of a range of soil properties under contrasting long-term management practices could result in a better understanding of the inter-relationships between soil microbial communities and soil biochemical properties that influence soil function and would result in a comprehensive assessment of soil quality [2]. Cover crop biomass is a precursor of the persistent organic matter that is a source of energy for microorganisms, and also affects the physicochemical properties of the soil [3]. Soil microbial biochemical activity is an indicator of the degradation and transformation of soil organic matter, but also provides vital information as to the quality of the soil [4]. Soil enzymes constantly play important roles in the maintenance of soil ecology and soil health. These enzymatic activities in the soil are mainly of microbial origin, and are derived from intracellular, cell-associated or free enzymes. Therefore, microorganisms can act as indicators of soil health as they actively affect nutritional cycling, and also affect the physical and chemical properties of the soil. Microorganisms respond rapidly to even slight changes by changing their population and activities, and thus, can be used for soil

health assessment. In contrast, soil enzymes are direct mediators for biological catabolism of soil organic and mineral components and they are often closely related to soil organic matter, soil physical properties, and microbial activities or biomass. Soil enzymes are better indicators of soil health as changes occur much sooner than other parameters, thereby providing early indications of changes in soil health [5].

Soil microorganisms increase the photosynthetic activity of plants, which results in better crop yields [6]. Previous studies have shown that the cultivation system significantly influences the crop and its physiological processes, i.e., photosynthetic activity, stomatal conductance, and transpiration rates [7]. Straw catch crop biomass is crucial for soil activity although the depth of cover of the plant biomass, its distribution and mixing with the topsoil layer is determined by soil cultivation treatments, which also directly and indirectly affect the physical, chemical, and biological properties of the soil.

Agrotechnical treatments (e.g., tillage system) affect the development of microorganisms and enzymatic activity in the soil. Bielińska and Mocek-Plóćiniak [8] suggest that both factors affect the amount of organic compounds that are biochemically mineralised and, consequently, the supply of nutrients to the plants. Natywa et al. [9] reported greater enzyme activity when traditional soil tillage was practiced, which may improve the soil condition at the optimum soil moisture and humidity levels.

Cover crops (CC) are a promising sustainable farming method that may improve the condition of the soil and mitigate its degradation. They can create a better agroecosystem than conventional tillage (ploughing) [10], and provide many ecosystem services, although not all the ancillary benefits are immediately measurable. For example, while CC do not always result in immediate increases in subsequent crop yields, their establishment provides vegetative cover, reduces wind and water erosion, and improves soil physical, chemical, and biological processes [11]. Moreover, by adding root biomass, CC can increase soil organic C concentrations and enhance microbial activity, which in turn can improve soil aggregation, aeration, water infiltration, porosity, and other soil physical processes and properties [12]. All these CC-induced improvements in soil processes are essential to sustainable agricultural production and environmental quality and deserve consideration. The activity of the soil microbiome is likely to increase after the use of CC and reduced tillage, which will be manifested by a greater number of microbial groups and enhanced enzymatic activity, as a reaction to the better conditions in the soil environment. Consequently, the yield of the main crops may potentially increase.

While there is a growing body of research on the effects of cover crop and crop cultivation methods on the activity of the soil microbiome (e.g., [13,14]), a sufficiently comprehensive and specific research synthesis to demonstrate all interactions that occur in the soil during the various phases of crop development (BBCH) has not been demonstrated to date.

Our 3-year experiment involved a meta-analysis, which compared the results of research on soil microbial activity (expressed by changes in the count of selected physiological microbial groups) and the activity of selected soil enzymes at different phases of wheat (*Triticum aestivum* L.) development. Methods of soil tillage and cover crop influence on soil microbial and enzymatic activity and depend on the phase of plant development. The analysis was conducted to determine the optimal effects of a combination of soil tillage methods on microbial activity under a cover crop and a spring wheat crop. Multivariate characterisation of phenotypic variability across 27 treatments (combinations of cover crop, tillage method, and year) was conducted. Canonical variate analysis (CVA) (based on the multivariate analysis of variance) was applied to the observations of nine microbiological traits in a split-plot design experiment.

2. Materials and Methods

2.1. A Field Experiment

Between 2011–2013, a field experiment was conducted at Brody Research and Education Station, Poznań University of Life Sciences, Poland (52°26' N; 16°17' E) on soils

classified as *Albic Luvisols* (WRB 2007), which have developed as loamy sands over loamy material (12% clay, 19% silt, and 69% sand). The random block (split-plot) design experiment was conducted with two experimental factors and four replications: The first-order factor was the effect of the stubble cover crop (white mustard cultivar *Nakielska*): Control variant: Zero (Z)—no cover crop sown, cover crop sown after skimming (S), and no tillage cover with the crop sown directly in the soil (DS). The second experimental factor was the spring cultivation tillage method: Direct sowing (DS), simplified tillage (ST) using a combined cultivator to a depth of 12–15 cm, and spring ploughing (PT) to a depth of 25 cm.

We tested the effects of the two experimental factors on nine microbiological traits: Dehydrogenase activity (DHA; mmol TPF kg⁻¹ dm soil 24 h⁻¹); acid phosphatase activity (PAC; mmol PNP kg⁻¹ dm soil h⁻¹); *Azotobacter* (cfu g⁻¹ dm soil); protease activity (PR; µg tyrosine g⁻¹ dm soil h⁻¹); moulds (cfu 10⁴ g⁻¹ dm soil); copiotrophic bacteria (cfu 10⁵ g⁻¹ dm soil); oligotrophic bacteria (cfu 10⁵ g⁻¹ dm soil); actinobacteria (cfu 10⁴ g⁻¹ dm soil); total bacterial count (cfu 10⁵ g⁻¹ dm soil).

Spring wheat cultivar Vinjett was sown at a rate of 400 seeds m⁻² across all tillage treatments. Each treatment plot was 10 m long and 4.5 m wide (45 m²). Spring wheat was sown on 25 March 2011, 23 March 2012, and 17 April 2013. Seeds were sown at a depth of 3–4 cm in all tillage treatments.

Identical fertilisation rates (90 kg N ha⁻¹, 26 kg P ha⁻¹, 50 kg K ha⁻¹) were applied to all tillage treatments and in each year of the experiment. The herbicide programme consisted of both pre-plant and post-emergence applications. Before sowing, 1.5 L ha⁻¹ of glyphosate herbicide +1.5 L ha⁻¹ of adjuvant AS 500 SL was applied to all the plots without tillage to control perennial weeds and volunteer plants. Lintur 70 WG (dicamba 65.9% + triasulfuron 4.1%) + Chwastox Extra 300 SL (MCPA 300 g L⁻¹) were applied at 150 g ha⁻¹ + 1.0 L ha⁻¹ during the growing season at BBCH 22 (post-emergence phase) for weed control. Falcon 460 EC fungicide (spiroxamine 250 g L⁻¹ + tebuconazole 167 g L⁻¹ + triadimenol 43 g L⁻¹) was applied at 0.6 L ha⁻¹ in all the plots at BBCH 32, and Fury 100 EW insecticide (zeta-cypermethrin 100 g L⁻¹) was applied at 0.1 L ha⁻¹. In the third and final year of the experiment, Karate Zeon 050 CS (lambda—cyhalotrin) was applied at 0.1 L ha⁻¹ at BBCH 61.

2.2. Sampling and Measurements

Soil samples for biochemical and microbiological analyses were collected from the arable layer (0–20 cm) at five terms that corresponded to the following phases of wheat development: 1st term—pre-sowing, 2nd term—tillering phase (BBCH 23), 3rd term—second node (BBCH 32), 4th term—heading (BBCH 55), and 5th term—post-harvest. The soil samples were randomly collected from nine treatments between the rows of each experimental treatment, with four replicates of each of the nine treatments. Thus, 20 (1 kg) samples of soil were collected during each term. They were placed into a ziplock bag and were transported in a refrigerator at 5 °C. Microbial analyses were performed immediately. The soil was sieved before microbiological and biochemical analyses.

2.3. Counts of Microorganisms and Analyses of Soil Enzyme Activity

Microbial count in the soil samples collected at a depth of 0–20 cm was determined by serial dilution on appropriate agars (with five replicates). The average count of the following colonies of microorganisms per dry mass of soil was measured:

- Total bacterial count—on ready-made Merck standard agar after 5 days of incubation at 25 °C;
- Moulds—on Martin agar [15] after 5 days of incubation at 24 °C;
- Copiotrophs—on nutrient agar (NA) [16] after 5 days of incubation at 25 °C;
- Oligotrophs—on diluted nutrient agar (DNA) [16] after 5 days of incubation at 25 °C;
- Actinobacteria—on Pochon agar after 5 days of incubation at 25 °C [17].

Soil DHA was measured by colorimetry (EC 1.1.1.) with 1% triphenyl tetrazolium chloride (TTC) as a substrate, after 24-h of incubation at 30 °C at a wavelength of 485 nm,

expressed as μmol triphenyl formazan (TPF) kg^{-1} $(24 \text{ h})^{-1}$ [18]. Soil PAC was measured (100 g of soil was taken from each experimental plot) with the method developed (EC 3.1.3.2) according to Tabatabai and Bremner [19]. The enzyme activity was determined with disodium p-nitrophenyl phosphate tetrahydrate, which was used as a substrate after 1 h of incubation at 37°C , at a wavelength of 400 nm. The results were converted into μmol (p-nitrophenol) PNP $\text{h}^{-1} \text{ g}^{-1} \text{ dm}$ (dry matter) of soil.

The protease activity (EC 3.4) was measured by means of the method developed by Ladd and Butler [20]. The enzyme activity was assessed by measuring the quantity of amino acids (tyrosine) formed, according to the following formula— μg tyrosine $\text{g}^{-1} \text{ DM}$ of soil 1 h^{-1} . In addition, 1% sodium caseinate was used as a substrate. Next, 5 mL of the substrate was added to 2 g of soil and incubated for 1 h at a temperature of 50°C in a vortex mixer (120 rpm). Then, 2 mL of 17.5% trichloroacetic acid (TCA) solution was added and the samples were cooled with ice to inhibit the activity of proteolytic bacteria. Next, the solution was filtered (90 mm paper filters) and 3 mL of 1.4 M NaCO_3 and 1 mL of Folin's reagent (incubated for 10 min) were added. The enzymatic activity was measured by spectrophotometry at a wavelength of 578 nm.

2.4. Weather Conditions

The characteristics of the growing seasons in terms of water and thermal conditions (Table 1) were assessed with the Sielianinov (k) hydrothermal coefficient (Figure 1), which was calculated as follows:

$$k = \frac{P}{0.1 \cdot \sum t}$$

where P is the sum of monthly precipitation (mm) and $\sum t$ is the sum of average daily air temperatures for a given month ($^\circ\text{C}$). The coefficient shows dry periods and optimal periods for plant growth [21]. The Sielianinov index indicates humidity levels during the growth of wheat throughout the experiment.

Table 1. Total rainfall and demand for rainfall.

Years	Total Rainfall (mm)			
	April	May	June	July
2011	13.9	34.0	15.4	175.4
2012	22.9	77.2	69.8	197.6
2013	15.4	163.0	125.3	67.3
Mean (1961–2010)	38.0	57.4	61.8	77.5
Monthly Demand for Rainfall (mm)				
Demand for rainfall	45	66	83	89

In the first year (2011), there was insufficient humidity for spring wheat until mid-May. There was also insufficient humidity in early June but excessive humidity in mid- and late July. Aside from these periods, conditions for the growth and development of spring wheat were good. In the second year of the experiment (2012), drought occurred in late April and in mid- and late May at the flag leaf stage of spring wheat. There was high moisture content in June and in early and mid-July. A semi-drought period also occurred at the beginning of July. Aside from these periods, moisture conditions for the development of spring wheat were good. In the third year (2013), droughts in mid-April, May, and June were compensated by rainfall at the end of those months.

2011

2012

2013

Figure 1. Sielianinov index values reflect the diversity in weather conditions in the years of the study (Interpretation: $K > 1.5$ indicates excessive moisture for all plants, $K = 1.0$ – 1.5 indicates sufficient moisture, $K = 0.5$ – 1.0 indicates insufficient moisture, and $K < 0.5$ indicates moisture less than the requirement for most plants (drought)). The black line indicates a lower limit of sufficient moisture. I, II and III—decades of the month.

2.5. Statistical Analysis

All statistical analyses were conducted for the five terms independently. Normality of the distributions of the traits under study was tested with Shapiro-Wilk's normality test [22]. Multivariate analysis of variance (MANOVA) was applied as follows:

$$Y = XT + E$$

where **Y** is the matrix of observations, **X** is the matrix of design, **T** is the matrix of unknown effects, and **E** is the matrix of residuals [23].

Next, the three-way analysis of variance (ANOVA) was carried out to determine the effects of cover crop, tillage method, and year (and their interactions) on the variability of the observed traits. The observed relationships were assessed with linear correlation coefficients. The results were also analysed with multivariate methods, such as CVA [24]. Mahalanobis distance [25] is suggested as a measure of similarity of multi-trait treatments [26], and was calculated for the treatments in the study. The GenStat v. 18 statistical software package was used for all the analyses.

3. Results

All the observed quantitative traits and multivariate factors were normally distributed. All the factors and their interactions (year $\times$ cover crop, year $\times$ tillage method, cover crop $\times$ tillage method, year $\times$ cover crop $\times$ tillage method) were significantly different for all nine traits at all five terms (Table 2). The statistically significant influence of year and year $\times$ cover crop interaction was observed for all nine traits at all five terms (Table 3). Cover crop was found to be significant for all traits, with the exception of copiotrophic bacteria at the 1st term, PAC at the 3rd term, and DHA at the 4th term (Table 3). The tillage method was significant for all traits, with the exception of PAC at the 1st and 5th terms, as well as *Azotobacter* and copiotrophic bacteria at the 1st term (Table 3). Year $\times$ tillage method interaction was significant for all traits, with the exception of PAC at the 1st term, PR at the 2nd term, and DHA at the 5th term (Table 3). Cover crop $\times$ tillage method interaction was significant for all traits, with the exception of copiotrophic bacteria (1st term), DHA and PAC (4th term). Year $\times$ cover crop $\times$ tillage method interaction was significant for all traits, with the exception of DHA at the 2nd term (Table 3). There were no interactions between some of the agronomic factors and microbiological parameters, e.g., the count of selected physiological microbial groups and the level of enzyme activity at all five terms.

Table 2. Results of multivariate analysis of variance (MANOVA) for field experiment conducted at Brody Research and Education Station, Poznań University of Life Sciences, Poland (52°26' N; 16°17' E) in 2011–2013. *** $p < 0.001$.

Term	1st Term before Sowing of Spring Wheat		2nd Term—Tillering Phase (BBCH 23)		3rd Term—2nd Node (BBCH 32)		4th Term—Heading (BBCH 55)		5th Term—Post-Harvest	
	Wilks' lambda	Rao F	Wilks' lambda	Rao F	Wilks' lambda	Rao F	Wilks' lambda	Rao F	Wilks' lambda	Rao F
Year (Y)	0.00004	1347 ***	0.00004	1355 ***	0.00134	213.4 ***	0.00004	1231 ***	0.00027	487.8 ***
Cover crop (Cc)	0.00475	109.6 ***	0.00381	123.3 ***	0.00083	273.4 ***	0.00472	110.0 ***	0.00746	85.8 ***
Tillage method (TM)	0.00672	90.82 ***	0.00832	80.84 ***	0.00289	142.7 ***	0.00826	81.16 ***	0.01038	71.52 ***
Y $\times$ Cc	0.00005	101.4 ***	0.00006	95.3 ***	0.00001	162.6 ***	0.00001	147.5 ***	0.00008	86.34 ***
Y $\times$ TM	0.00026	61.91 ***	0.00052	49.84 ***	0.00006	95.46 ***	0.00013	75.76 ***	0.00233	30.93 ***
Cc $\times$ TM	0.00014	74.39 ***	0.00024	62.79 ***	0.00001	169.3 ***	0.00004	104.4 ***	0.00058	48.25 ***
Y $\times$ Cc $\times$ TM	0	71.51 ***	0	42.11 ***	0	59.87 ***	0	63.98 ***	0	39.52 ***

Table 3. F-statistic from three-way analysis of variance for the traits observed at the five terms.

Term	Source of Variation	Year (Y)	Cover Crop (Cc)	Tillage Method (TM)	Y × Cc	Y × TM	Cc × TM	Y × Cc × TM
	d.f.	2	2	2	4	4	4	8
I	DHA	219.33 ***	15.14 ***	26.5 ***	19.1 ***	16.08 ***	7.1 ***	3.88 ***
	PAC	624.79 ***	9.97 ***	0.53	2.71 *	2.4	4.91 **	3.95 ***
	<i>Azotobacter</i>	300.33 ***	15.4 ***	1.47	12.17 ***	5.88 ***	13.71 ***	4.39 ***
	PR	106.19 ***	21.13 ***	16.73 ***	82.56 ***	102.15 ***	361.81 ***	330.33 ***
	Moulds	2368.83 ***	40.72 ***	130.37 ***	45.47 ***	102.28 ***	80.48 ***	141.38 ***
	Copiotrophic bacteria	13.57 ***	3.08	1.68	2.97 *	2.74 *	1.43	2.17 *
	Oligotrophic bacteria	4648.49 ***	588.09 ***	233.67 ***	798.11 ***	126.55 ***	204.84 ***	377.75 ***
	Actinobacteria	3761.66 ***	19.05 ***	257.02 ***	117.02 ***	38.49 ***	67.59 ***	328.35 ***
	Total bacterial count	2500.69 ***	587.71 ***	209.53 ***	319.86 ***	316.96 ***	74.96 ***	221 ***
II	DHA	41.37 ***	5.99 **	17.78 ***	9.65 ***	5.07 **	3.99 **	0.95
	PAC	957.61 ***	11.45 ***	8.43 ***	2.59 *	3.11 *	3.94 **	2.45 *
	<i>Azotobacter</i>	627.97 ***	41.91 ***	27.37 ***	63.43 ***	18.79 ***	50.07 ***	23.34 ***
	PR	92.9 ***	242.42 ***	450.42 ***	553.58 ***	154.86 ***	109.46 ***	387.7 ***
	Moulds	1155.73 ***	202.18 ***	57.77 ***	149.13 ***	48.04 ***	79.33 ***	68.58 ***
	Copiotrophic bacteria	1860.08 ***	80.08 ***	66.99 ***	85.48 ***	116.94 ***	76.9 ***	41.16 ***
	Oligotrophic bacteria	2356.37 ***	329.44 ***	13.44 ***	322.56 ***	35.95 ***	19.11 ***	73.74 ***
	Actinobacteria	3354.9 ***	141.96 ***	346.29 ***	125.08 ***	98.17 ***	213.08 ***	69.76 ***
	Total bacterial count	869.37 ***	97.67 ***	53.1 ***	3.76 **	85.23 ***	116.78 ***	39.55 ***
III	DHA	173.87 ***	3.61 *	4.75 *	2.52 *	5.63 ***	12.82 ***	7.16 ***
	PAC	121.49 ***	0.51	15.18 ***	3.4 *	4.04 **	6.18 ***	10.65 ***
	<i>Azotobacter</i>	319.79 ***	208.53 ***	91.58 ***	164.94 ***	151.36 ***	273.66 ***	107.44 ***
	PR	142.9 ***	217.11 ***	489.77 ***	254.67 ***	2.06	363.89 ***	41.93 ***
	Moulds	18.35 ***	98.29 ***	32.76 ***	74.73 ***	32.1 ***	27.08 ***	49.27 ***
	Copiotrophic bacteria	1141.73 ***	622 ***	371.21 ***	561.65 ***	522.67 ***	183.65 ***	185.84 ***
	Oligotrophic bacteria	516.58 ***	22.4 ***	174.62 ***	187.85 ***	36.16 ***	129.73 ***	211.85 ***
	Actinobacteria	103.57 ***	18.96 ***	107.02 ***	511.66 ***	150.9 ***	275.48 ***	94.46 ***
	Total bacterial count	413.62 ***	1013.95 ***	61.15 ***	111.19 ***	245.4 ***	285.4 ***	239.12 ***
IV	DHA	101.28 ***	0.82	12.93 ***	10.18 ***	10.5 ***	1.43	3.65 **
	PAC	679.34 ***	6.09 **	4.64 *	14.81 ***	9.44 ***	2.29	5.28 ***
	<i>Azotobacter</i>	1357.72 ***	42.39 ***	18.16 ***	58.19 ***	3.1 *	47.01 ***	106.56 ***
	PR	134.57 ***	402.16 ***	210.58 ***	484.22 ***	120.46 ***	390.36 ***	72.31 ***
	Moulds	3670.45 ***	296.67 ***	104.09 ***	242.56 ***	257.32 ***	316.32 ***	298.66 ***
	Copiotrophic bacteria	50.51 ***	10.24 ***	12.04 ***	95.38 ***	38.35 ***	31.1 ***	34.27 ***
	Oligotrophic bacteria	251.64 ***	206.29 ***	107.73 ***	378.18 ***	239.23 ***	315.98 ***	44.97 ***
	Actinobacteria	2973.18 ***	362.64 ***	291.05 ***	197.92 ***	64.42 ***	436.18 ***	94.3 ***
	Total bacterial count	1100.32 ***	17.15 ***	132.76 ***	112.01 ***	40.25 ***	50.72 ***	165.16 ***
V	DHA	26.34 ***	10.85 ***	10.47 ***	6.67 ***	0.78	5.06 **	2.56 *
	PAC	537 ***	3.56 *	1.17	9.67 ***	14.44 ***	15.26 ***	15.96 ***
	<i>Azotobacter</i>	23.69 ***	3.54 *	18.3 ***	41.62 ***	20.25 ***	11.47 ***	18.65 ***
	PR	456.91 ***	415.18 ***	304.43 ***	51.11 ***	80.45 ***	146.34 ***	16.98 ***
	Moulds	213.75 ***	53.13 ***	72.2 ***	54.75 ***	66.44 ***	10.39 ***	73.45 ***
	Copiotrophic bacteria	259.03 ***	15.94 ***	48.5 ***	23.63 ***	35.66 ***	10.25 ***	17.56 ***
	Oligotrophic bacteria	337.75 ***	34.15 ***	411.79 ***	237.82 ***	4.15 **	207.19 ***	304.04 ***
	Actinobacteria	2155.41 ***	416.72 ***	31.68 ***	363.97 ***	12.09 ***	29.26 ***	38.19 ***
	Total bacterial count	2165.47 ***	99.64 ***	19.75 ***	270.38 ***	125.82 ***	153.33 ***	188.13 ***

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; d.f.—number of degrees of freedom. DHA—dehydrogenases activity, PAC—acid phosphatase activity, PR—protease activity.

Statistically significant interdependencies were observed between spring wheat traits at the five terms (Table 4). Only two pairs of traits (moulds and copiotrophic bacteria, and oligotrophic bacteria and actinobacteria) were significantly correlated (positively or negatively) at all five terms. *Azotobacter* and PR, and copiotrophic bacteria and PR were not significantly correlated at the five terms (Table 4). In total, there were 102 pairs of significantly correlated coefficients in our experiment: 63 positive and 39 negative (Table 4). The Pearson test confirmed the relationships between the physiological microbial groups that occur in the soil after the application of organic matter.

Table 4. Correlation coefficients between the quantitative traits of spring wheat observed at the five terms. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Trait	Term	DHA	PAC	<i>Azotobacter</i>	PR	Moulds	Copiotrophic Bacteria	Oligotrophic Bacteria	Actinobacteria
PAC	I	0.62 ***							
	II	−0.42 ***							
	III	0.17							
	IV	−0.51 ***							
	V	−0.36 ***							
<i>Azotobacter</i>	I	0.27 **	0.54 ***						
	II	0.20 *	−0.56 ***						
	III	−0.11	−0.27 **						
	IV	−0.04	−0.30 **						
	V	−0.40 ***	0.07						
PR	I	0.12	−0.12	−0.08					
	II	0.30 **	0.02	0.14					
	III	−0.05	0.1	0.08					
	IV	0.06	0.21 *	−0.04					
	V	−0.05	0.41 ***	0					
Moulds	I	−0.46 ***	−0.14	0.43 ***	−0.24 *				
	II	0.15	0.28 **	0	−0.16				
	III	0.09	0.25 *	0.20 *	0.41 ***				
	IV	0.38 ***	−0.60 ***	0.04	−0.33 ***				
	V	−0.12	0.32 ***	−0.08	0.36 ***				
Copiotrophic bacteria	I	−0.23 *	−0.05	0.32 ***	0.01	0.50 ***			
	II	0.17	−0.12	0.53 ***	−0.02	0.39 ***			
	III	−0.24 *	−0.38 ***	−0.15	−0.11	−0.20 *			
	IV	−0.09	0.25 **	0.20 *	0.13	−0.28 **			
	V	0.45 ***	−0.54 ***	−0.07	−0.17	−0.38 ***			
Oligotrophic bacteria	I	−0.46 ***	−0.32 ***	0.07	−0.22 *	0.72 ***	0.29 **		
	II	0.35 ***	−0.34 ***	0.63 ***	0.08	0.27 **	0.70 ***		
	III	0.44 ***	0.14	0.01	0.20 *	0.32 ***	−0.24 *		
	IV	0.16	−0.20 *	0.14	−0.01	0.20 *	−0.11		
	V	−0.06	0.21 *	−0.16	0.14	0.14	0.18		
Actinobacteria	I	−0.49 ***	−0.75 ***	−0.42 ***	−0.13	0.26 **	0.09	0.44 ***	
	II	−0.06	0.52 ***	0	−0.02	0.53 ***	0.50 ***	0.38 ***	
	III	0.24 *	−0.04	0.06	0.28 **	0.28 **	0.11	0.39 ***	
	IV	−0.22 *	−0.01	0.49 ***	−0.08	−0.1	−0.01	0.52 ***	
	V	0.29 **	−0.40 ***	0.05	−0.35 ***	−0.32 ***	0.33 ***	−0.24 *	
Total bacterial count	I	−0.50 ***	−0.36 ***	0.14	0	0.66 ***	0.57 ***	0.55 ***	0.41 ***
	II	0.03	0.42 ***	−0.02	−0.09	0.64 ***	0.54 ***	0.34 ***	0.75 ***
	III	0.23 *	0.11	0	0.12	−0.01	−0.13	0.19	0.14
	IV	−0.23 *	0.04	0.51 ***	−0.06	−0.21 *	0.36 ***	0.29 **	0.73 ***
	V	0.16	−0.37 ***	0.13	−0.33 ***	−0.35 ***	0.36 ***	−0.07	0.77 ***

In our study, the individual traits differed in significance, and in their proportion of the total multivariate variation. In CVA, the first two canonical variates jointly explained between 55.53% (3rd term) and 74.79% (1st term) of the total variation observed between the treatments (Table 5, Figures 2–6). The most significant linear relationship with the first canonical variate (at the 1st term) was found for moulds, copiotrophic bacteria, oligotrophic bacteria, actinobacteria, total bacterial count (positive dependencies), DHA and PAC (negative dependencies) (Table 5). The second canonical variate was significantly positively correlated with PR and actinobacteria but negatively correlated with PAC and oligotrophic bacteria. There was a significant positive linear relationship with the first canonical variate at the 2nd term for moulds, copiotrophic bacteria, oligotrophic bacteria, actinobacteria, and total bacterial count (Table 5). The second canonical variate at the 2nd term was significantly positively correlated with PAC and PR but negatively correlated with *Azotobacter*, copiotrophic bacteria and oligotrophic bacteria. At the 3rd term, there was only one positive correlation between the first two canonical variates and the original traits: Copiotrophic bacteria (for the first canonical variate), and PR, moulds, oligotrophic bacteria and actinobacteria (for the second canonical variate). *Azotobacter*, actinobacteria

and the total bacterial count were correlated with the first canonical variate at the 4th term, whereas DHA, PAC, PR, moulds, and oligotrophic bacteria were correlated with the second canonical variate. There was a significant linear relationship with the first canonical variate at the 5th term for PAC, PR, oligotrophic bacteria (positive dependencies), as well as actinobacteria and total bacterial count (negative dependencies). The second canonical variate was significantly negatively correlated with oligotrophic bacteria (Table 5). The traits exhibited differing levels of activity at the five terms, most likely caused by the weather conditions. Year was found to be the main differentiating factor (Figure 3), as soil moisture and the amount of rainfall had a highly significant influence on microbial count and their biochemical activity.

Table 5. Correlation coefficients between the first two canonical variates and the original traits at the five terms.

Term	I		II		III		IV		V	
	V ₁	V ₂	V ₁	V ₂	V ₁	V ₂	V ₁	V ₂	V ₁	V ₂
Trait										
DHA	−0.66 ***	−0.07	0.16	−0.16	−0.06	0.24	−0.3	0.39 *	−0.34	−0.02
PAC	−0.55 **	−0.40 *	0.35	0.68 ***	−0.31	0.07	0.13	−0.68 ***	0.57 **	0.02
<i>Azotobacter</i>	0.03	−0.34	0.24	−0.61 ***	−0.34	0.23	0.65 ***	0.27	−0.06	0.22
PR	−0.15	0.39 *	0.16	0.55 **	0	0.61 ***	0.19	−0.42 *	0.44 *	0.06
Moulds	0.79 ***	−0.33	0.66 ***	−0.05	−0.15	0.61 ***	−0.35	0.91 ***	0.38	−0.13
Copiotrophic bacteria	0.69 ***	0.09	0.73 ***	−0.50 **	0.93 ***	−0.18	0.27	−0.28	−0.34	−0.28
Oligotrophic bacteria	0.82 ***	−0.51 **	0.63 ***	−0.59 **	0	0.58 **	0.37	0.47 *	0.40 *	−0.87 ***
Actinobacteria	0.66 ***	0.39 *	0.89 ***	0.16	0.35	0.73 ***	0.88 ***	0.28	−0.93 ***	−0.05
Total bacterial count	0.88 ***	0.22	0.83 ***	0.05	0.02	−0.21	0.88 ***	0.12	−0.88 ***	−0.36
Variation percentage	60.15	14.64	54.26	18.84	27.96	27.57	39.27	30.7	53.03	20.65

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. V₁—first canonical variate; V₂—second canonical variate.

Figure 2. Distribution of the spring wheat treatments in the two first canonical variates at the 1st term (cover crop: Z—zero, S—skimming, DS—direct sowing, ST—simplified tillage, PT—spring ploughing; years: 1—2011, 2—2012, 3—2013).

Figure 3. Distribution of the spring wheat treatments in the two first canonical variates at the 2nd term (cover crop: Z—zero, S—skimming, DS—direct sowing, ST—simplified tillage, PT—spring ploughing; years: 1—2011, 2—2012, 3—2013).

Figure 4. Distribution of the spring wheat treatments in the two first canonical variates at the 3rd term (cover crop: Z—zero, S—skimming, DS—direct sowing, ST—simplified tillage, PT—spring ploughing; years: 1—2011, 2—2012, 3—2013).

Figure 5. Distribution of the spring wheat treatments in the two first canonical variates at the 4th term (cover crop: Z—zero, S—skimming, DS—direct sowing, ST—simplified tillage, PT—spring ploughing; years: 1—2011, 2—2012, 3—2013).

Figure 6. Distribution of spring wheat treatments in the two first canonical variates at the 5th term (cover crop: Z—zero, S—skimming, DS—direct sowing, ST—simplified tillage, PT—spring ploughing; years: 1—2011, 2—2012, 3—2013).

The greatest diversity in all nine traits (measured by Mahalanobis distance) at the five terms was observed for the following treatments: 1st term—DS-DS-1 (cover crop sown directly, wheat sown directly) and DS-ST-2 (cover crop sown directly, wheat sown into soil tilled with a combined cultivator) (Mahalanobis distance was 84.10); 2nd term—DS-ST-1 and Z-ST-2 (63.18); 3rd term—DS-PT-1 and DS-DS-2 (62.31); 4th term—DS-ST-1 and Z-PT-2 (70.02); 5th term—S-ST-1 and Z-DS-3 (55.87). The following treatments exhibited the greatest similarities: 1st term—Z-PT-1 (without cover crop, wheat sown after ploughing) and S-ST-1 (cover crop sown after skimming, wheat sown into soil tilled with a combined cultivator) (3.32); 2nd term—Z-ST-1 and DS-DS-1 (7.05); 3rd term—Z-PT-1 and S-ST-1 (3.68); 4th term—S-PT-1 and DS-PT-1 (5.39); 5th term—Z-ST-2 and S-DS-2 (5.70). The density of the Mahalanobis distance at the five terms is shown in Figure 7. Phenotypic diversity (measured by Mahalanobis distance) was significant and positively correlated at the following terms: 1st and 2nd, 1st and 3rd, 1st and 4th, 2nd and 4th, 2nd and 5th (Table 6, Figure 8). However, it was negatively correlated at the 3rd and 4th terms, and 3rd and 5th terms (Table 6, Figure 8).

Figure 7. Density of Mahalanobis distance at the five terms: I—1st term—pre-sowing, II—2nd term—tillering phase (BBCH 23), III—3rd term—second node (BBCH 32), IV—4th term—heading (BBCH 55), and V—5th term—post-harvest.

Table 6. Correlation coefficients between Mahalanobis distance, estimated at the five terms.

Term	I	II	III	IV	V
I	1				
II	0.3011 ***	1			
III	0.1176 *	−0.0492	1		
IV	0.2258 ***	0.511 ***	−0.1195 *	1	
V	−0.0261	0.1079 *	−0.1988 ***	−0.0211	1

* $p < 0.05$; *** $p < 0.001$. **Figure 8.** Heatmaps for linear Pearson's correlation coefficients between Mahalanobis distance, estimated for all pairs of treatments at the five terms. I—1st term—pre-sowing, II—2nd term—tillering phase (BBCH 23), III—3rd term—second node (BBCH 32), IV—4th term—heading (BBCH 55), and V—5th term—post-harvest.

4. Discussion

Many of the earlier studies that have examined the influence of soil cultivation systems and the use of cover crops showed that they significantly affect soil microbial activity, i.e., metabolic activity (DHA, acid phosphatase, urease) and lead to changes in the composition of soil microbiota (copiotrophs, oligotrophs and actinobacteria) at the phylum level [6,27]. In contrast to the physical and chemical properties of the soil, which tend to change rather slowly, the biological properties of the soil are more sensitive to changes in its use [28]. Long-term experiments are critical to observe all the interactions that occur when cover crops are grown under various cultivation systems, and the effects of the different methods on the growth of a specific crop. The results of our experiment are in line with the meta-analysis conducted by Kim et al. [29], who evaluated the influence of cover crops on various microbiological parameters. Those researchers found that weather conditions (in our study—year of the research), cover crops and cultivation method significantly influence the level of microbial activity in the soil. The lack of interactions between some agronomic factors and microbiological parameters in our study could be explained by the fact that the increase in the soil microbial activity may be associated with an increase in the count of selected physiological microbial groups, it is not always related to the level of enzymes produced extracellularly by microorganisms or vice versa. The production of extracellular enzymes is variable and depends on the physiological microbial group and is not universal, especially the activity of the soil microorganisms that are responsible for ecosystem services, such as the circulation of nutrients [30]. However, the links between the physiological groups of soil microorganisms, the specific enzymes they produce, and genomic data is not clear.

The positive correlation between moulds and copiotrophs at all five terms in our study can be explained by the fact that both groups of microorganisms become active in the soil mainly when there is an inflow of fresh organic matter, and grow rapidly when there is an abundance of nutrients. It is assumed that copiotrophs (such as moulds) are able to adapt to a wide range of habitats and niches, which means that they are generalists [31]. Oligotrophic bacteria and actinobacteria exhibit similar properties, both groups belong to the so-called group of organic bacteria, and are indicative of soil fertility. Oligotrophic bacteria are able to grow despite a scarcity of nutrients and are more specialised in terms of the substrate in the environment. Actinobacteria affect soil fertility and participate in the circulation of elements. However, their high activity in the soil, manifested by an increased count, indicates a reduced water content. In our study, the most important traits in the multivariate analyses were identified by CVA [32,33], where the observed dependencies showed that the agrotechnical treatments (use of cover crops and various methods of cultivation) increased the count of soil microorganisms. However, the increase in count was not always closely correlated with the pool of enzymes they produced. The cultivation of cover crops increased root secretions in the soil, and thus accelerated the proliferation of microorganisms. Daryanto et al. [34] observed similar relationships in their study. It should be noted, however, that the pool of active soil enzymes is affected by secretions from the root system and by the demand of the crop for specific nutrients at a particular stage of its development (BBCH). The substances contained in the root secretions and in the dying cells of the root tissues are a rich source of nutrients and energy for various physiological groups of microorganisms. Indeed, the study by Hupe et al. [35] showed that the developmental phase of the plant significantly influences the dynamics of nutrients in the root zone and, thus, soil enzymatic activity. Those researchers observed substantial deposition of C and nitrogen in the rhizosphere in the period from emergence to flowering. They also stressed that the amount of nitrogen deposited in the rhizosphere was significantly inhibited after the flowering phase, which was caused by the displacement of nitrogen in the plants to yield components (i.e., grain, straw). The decrease in the amount of organic nitrogen substances in the rhizosphere in relation to C after flowering may explain the reduced metabolism of some soil enzymes in relation to the count of selected physiological microbial groups. Plant emergence and flowering phases are times

of very high demand for phosphorus. During these phases, there is usually increased PAC activity in the soil. If there is low availability of phosphorus in the soil, there is greater PAC secretion, not only by the communities of soil microorganisms but also by the plant itself. This significantly increases the pool of this enzyme in the soil pedon, which is often negatively correlated with the microbial count.

In turn, Piazza et al. [36] conducted a similar study on the relationship between conservation tillage and the diversity of microorganisms and soil enzymatic parameters and noticed that soil enzymatic parameters are significantly related only to the structure of fungal communities along the soil profile, but not to the diversity of prokaryotic communities.

Many studies indicate that weather conditions significantly affect soil microbiological activity (e.g., [37–39]). The water content in the soil affects the physiological state of microorganisms and plants [40,41]. Soils with a high moisture content hold more functionally diverse microbial communities. Drought may also disturb soil homeostasis [42,43]. In addition, water is essential for soil enzymes to maintain catalytic activity [44,45].

5. Conclusions

In our study, year was the main factor differentiating soil microbial count and activity. Soil moisture and the amount of rainfall strongly determined the biochemical activity. The traits (moulds and copiotrophic bacteria, as well as oligotrophic bacteria and actinobacteria) were significantly correlated (positively or negatively) at all five terms. Pearson's test confirmed the relationships between the physiological groups of soil microorganisms after the application of organic matter, and captured the positive correlation between moulds and copiotrophs at all five terms of the study. The 27 combinations of cover crops, tillage methods, and years were different distributed in the two first canonical variates at the studied terms. Phenotypic diversity was significant and positively correlated between pre-sowing and other terms except post-harvest term.

Piazza et al. [36] conducted a similar research on dependencies between conservation tillage and microorganism diversity.

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References

1. Al-Kaisi, M.M.; Yin, X.; Licht, M.A. Soil carbon and nitrogen changes as affected by tillage system and crop biomass in a corn-soybean rotation. *Appl. Soil Ecol.* **2005**, *30*, 174–191. [[CrossRef](#)]
2. Mbuthia, L.W.; Martínez, V.A.; Schaeffer, J.S.D.; Tyler, D.; Odoi, E.; Mpheshea, M.; Walker, F.; Eash, N. Long term tillage, cover crop, and fertilization effects on microbial community structure, activity: Implications for soil quality. *Soil Biol. Biochem.* **2015**, *89*, 24–34. [[CrossRef](#)]

3. Blanco-Canqui, H.; Shaver, T.M.; Lindquist, J.L.; Shapiro, C.A.; Elmore, R.W.; Francis, C.A.; Hergert, G.W. Cover crops and ecosystem services: Insights from studies in temperate soils. *Agron. J.* **2015**, *107*, 2449–2474. [\[CrossRef\]](#)
4. Veum, K.S.; Goyne, K.W.; Kremer, R.J.; Miles, R.J.; Sudduth, K.A. Biological indicators of soil quality and soil organic matter characteristics in an agricultural management continuum. *Biogeochemistry* **2014**, *117*, 81–99. [\[CrossRef\]](#)
5. Das, S.K.; Varma, A. Role of enzymes in maintaining soil health. In *Soil Enzymology*; Springer: Berlin/Heidelberg, Germany, 2010; pp. 25–42.
6. Niewiadomska, A.; Majchrzak, L.; Borowiak, K.; Wolna-Maruwka, A.; Waraczewska, Z.; Budka, A.; Gaj, R. The influence of tillage and cover cropping on soil microbial parameters and spring wheat physiology. *Agronomy* **2020**, *10*, 200. [\[CrossRef\]](#)
7. Yang, Y.; Ding, J.; Zhang, Y.; Wu, J.; Zhang, J.; Pan, X.; Gao, C.; Wang, Y.; He, F. Effects of tillage and mulching measures on soil moisture and temperature, photosynthetic characteristics and yield of winter wheat. *Agric. Water Manag.* **2018**, *201*, 299–308. [\[CrossRef\]](#)
8. Bielińska, E.J.; Mocek-Płóćiniak, A. Wpływ systemu uprawy na aktywność enzymatyczną gleby. *Arch. Environ. Prot.* **2012**, *38*, 75–82. [\[CrossRef\]](#)
9. Natywa, M.; Majchrzak, L.; Sawicka, A. Wpływ sposobu uprawy roli na aktywność enzymatyczną gleby i plon kukurydzy. *Ekol. Tech.* **2009**, *17*, 171–177.
10. Six, J.; Fray, S.D.; Thiet, R.K.; Batten, R.K. Bacterial and Fungal Contributions to Carbon Sequestration in Agroecosystems. *Soil Sci. Soc. Am. J.* **2006**, *70*, 555–559. [\[CrossRef\]](#)
11. Blanco-Canqui, H.; Holman, J.D.; Schlegel, A.J.; Tatarko, J.; Shaver, T.M. Replacing fallow with cover crops in a semiarid soil: Effects on soil properties. *Soil Sci. Soc. Am. J.* **2013**, *77*, 1026–1034. [\[CrossRef\]](#)
12. Blanco-Canqui, H.; Mikha, M.M.; Presley, D.R.; Claassen, M.M. Addition of cover crops enhances no-till potential for improving soil physical properties. *Soil Sci. Soc. Am. J.* **2011**, *75*, 1471–1482. [\[CrossRef\]](#)
13. Cerecetto, V.; Smalla, K.; Nesme, J.; Garaycochea, S.; Fresia, P.; Sørensen, S.; Babi, D.; Leoni, C. Reduced tillage, cover crops and organic amendments affect soil microbiota and improve soil health in Uruguayan vegetable farming systems. *FEMS Microbiol. Ecol.* **2021**, *97*, fiab023. [\[CrossRef\]](#)
14. Adetunji, A.T.; Ncube, B.; Mulidzi, R.; Lewu, F.B. Management impact and benefit of cover crops on soil quality: A review. *Soil Tillage Res.* **2020**, *204*, 104717. [\[CrossRef\]](#)
15. Martin, J.P. Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. *Soil Sci.* **1950**, *69*, 215–232. [\[CrossRef\]](#)
16. Ohta, H.; Hattori, T. Bacteria sensitive to nutrient broth medium in terrestrial environments. *Soil Sci. Plant Nutr.* **1980**, *26*, 99–107. [\[CrossRef\]](#)
17. Grabińska-Łoniewska, A. *Laboratory Classes in General Microbiology*; Oficyna Wydawnicza Politechniki: Warsaw, Poland, 1999.
18. Thalmann, A. Zur Methodik der Bestimmung der Dehydrogenase aktivität im Boden mittels triphenyltetrazoliumchlorid (TTC). *Landwirtsch. Forsch.* **1968**, *21*, 249–258.
19. Tabatabai, M.A.; Bremner, J.M. Use of *p*-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol. Biochem.* **1969**, *1*, 301–307. [\[CrossRef\]](#)
20. Ladd, J.N.; Butler, J.H.A. Humus–enzyme systems and synthetic organic polymer–enzyme analogs. In *Soil Biochemistry*; Paul, E.A., McLaren, A.D., Eds.; Marcel Dekker: New York, NY, USA, 1975; pp. 143–193.
21. Stachowski, P. Evaluation of meteorological droughts in post-mining areas in Poland in Konin area. Central Pomeranian Scientific Society for Environmental Protection. *Ann. Set. Environ. Protect.* **2010**, *12*, 587–606.
22. Shapiro, S.S.; Wilk, M.B. An analysis of variance test for normality (complete samples). *Biometrika* **1965**, *52*, 591–611. [\[CrossRef\]](#)
23. Bocianowski, J.; Majchrzak, L. Analysis of effects of cover crop and tillage method combinations on the phenotypic traits of spring wheat (*Triticum aestivum* L.) using multivariate methods. *Appl. Ecol. Environ. Res.* **2019**, *17*, 15267–15276. [\[CrossRef\]](#)
24. Rencher, A.C. Interpretation of canonical discriminant functions, canonical variates, and principal components. *Am. Stat.* **1992**, *46*, 217–225.
25. Mahalanobis, P.C. On the generalized distance in statistics. *Proc. Natl. Inst. Sci.* **1936**, *12*, 49–55.
26. Seidler-Łożykowska, K.; Bocianowski, J. Evaluation of variability of morphological traits of selected caraway (*Carum carvi* L.) genotypes. *Ind. Crop. Prod.* **2012**, *35*, 140–145. [\[CrossRef\]](#)
27. Swędryńska, D.; Małecka-Jankowiak, I. The Impact of Tillaging Spring Barley on Selected Chemical, Microbiological, and Enzymatic Soil Properties. *Pol. J. Environ. Stud.* **2017**, *26*, 303–313. [\[CrossRef\]](#)
28. Yao, R.J.; Yang, J.S.; Zhang, T.J.; Gao, P.; Yu, S.P.; Wang, X.P. Short-term effect of cultivation and crop rotation systems on soil quality indicators in a coastal newly reclaimed farming area. *J. Soils Sediments* **2013**, *13*, 1335–1350. [\[CrossRef\]](#)
29. Kim, N.; Zabaloy, M.C.; Guan, K.; Villamil, M.B. Do cover crops benefit soil microbiome? A meta-analysis of current research. *Soil Biol. Biochem.* **2020**, *142*, 107701. [\[CrossRef\]](#)
30. Wang, Z.; Li, T.; Wen, X.; Liu, Y.; Han, J.; Liao, Y.; DeBruyn, J.M. Fungal communities in rhizosphere soil under conservation tillage shift in response to plant growth. *Front. Microbiol.* **2017**, *8*, 1301. [\[CrossRef\]](#)
31. Kurm, V.; van der Putten, W.H.; de Boer, W.; Naus-Wiezer, S.; Hol, W.H.G. Low abundant soil bacteria can be metabolically versatile and fast growing. *Ecology* **2017**, *98*, 555–564. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Bocianowski, J.; Szulc, P.; Nowosad, K. Analysis of effects of nitrogen and magnesium fertilization combinations of phenotypic traits of two maize (*Zea mays* L.) cultivars using multivariate methods. *Pol. J. Agron.* **2016**, *26*, 3–8.

33. Lahuta, L.B.; Ciak, M.; Rybiński, W.; Bocianowski, J.; Börner, A. Diversity of the composition and content of soluble carbohydrates in seeds of the genus *Vicia* (Leguminosae). *Genet. Resour. Crop Evol.* **2018**, *65*, 541–554. [[CrossRef](#)]
34. Daryanto, S.; Fu, B.; Wang, L.; Jacinthe, P.A.; Zhao, W. Quantitative synthesis on the ecosystem services of cover crops. *Earth Sci. Rev.* **2018**, *185*, 357–373. [[CrossRef](#)]
35. Hupe, A.; Schulz, H.; Bruns, C.; Haase, T.; Heß, J.; Joergensen, R.G.; Wichern, F. Even flow? Changes of carbon and nitrogen release from pea roots over time. *Plant Soil* **2018**, *431*, 143–157. [[CrossRef](#)]
36. Piazza, G.; Ercoli, L.; Nuti, M.; Pellegrino, E. Interaction between conservation tillage and nitrogen fertilization shapes prokaryotic and fungal diversity at different soil depths: Evidence from a 23-year field experiment in the mediterranean area. *Front. Microbiol.* **2019**, *10*. [[CrossRef](#)]
37. Kardol, P.; Cregger, M.A.; Company, C.E.; Classen, A.T. Soil ecosystem functioning under climate change: Plant species and community effects. *Ecology* **2010**, *91*, 767–781. [[CrossRef](#)]
38. Yuan, B.C.; Li, Z.Z.; Liu, H.; Gao, M.; Zhang, Y.Y. Microbial biomass and activity in salt affected soils under arid conditions. *Appl. Soil Ecol.* **2007**, *35*, 319–328. [[CrossRef](#)]
39. Borowik, A.; Wyszowska, J. Soil moisture as a factor affecting the microbiological and biochemical activity of soil. *Plant Soil Environ.* **2016**, *62*, 250–255. [[CrossRef](#)]
40. Walker, T.S.; Bais, H.P.; Grotewold, E.; Vivanco, J.M. Root exudation and rhizosphere biology. *Plant Physiol.* **2003**, *132*, 44–51. [[CrossRef](#)] [[PubMed](#)]
41. Wyszowska, J.; Borowik, A.; Olszewski, J.; Kucharski, J. Soil bacterial community and soil enzyme activity depending on the cultivation of *Triticum aestivum*, *Brassica napus*, and *Pisum sativum* ssp. *arvense*. *Diversity* **2019**, *11*, 246. [[CrossRef](#)]
42. Kim, S.Y.; Lee, S.H.; Freeman, C.; Fenner, N.; Kang, H. Comparative analysis of soil microbial communities and their responses to the short-term drought in bog, fen, and riparian wetlands. *Soil Biol. Biochem.* **2008**, *40*, 2874–2880. [[CrossRef](#)]
43. Kumar, P. Significance of soil-root system and aquaporins for water homeostasis in plant—a review. *Adv. Plant Physiol.* **2014**, *15*, 324.
44. Ahmadi, A.; Baker, D. The effect of water stress on the activities of key regulatory enzymes of the sucrose to starch pathway in wheat. *Plant Growth Regul.* **2001**, *35*, 81–91. [[CrossRef](#)]
45. Jiang, M.G.; Zhang, J.H. Water stress-induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and up-regulates the activities of antioxidant enzymes in maize leaves. *J. Exp. Bot.* **2002**, *53*, 2401–2410. [[CrossRef](#)] [[PubMed](#)]