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Effects of growing spring barley in organic agriculture as a result of constructed bacterial consortia and living mulch

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Abstract. The objective of the research conducted in a temperate climate was to determine the effect of bacterial consortia in spring barley cultivated with living mulch on grain yield, grain yield structure, and the biological index of soil fertility. The experiment was conducted with the following two factors: bacterial consortia: control, inoculation with *Azospirillum lipoferum* Br17 and *Azotobacter chroococcum*, inoculation with *Pseudomonas fluorescens*, *Bacillus subtilis*, and *Bacillus amyloliquefaciens*, and inoculation with *Azotobacter chroococcum, Pseudomonas fluorescens*, *Bacillus subtilis*, and *Bacillus amyloliquefaciens*; living mulch: control, red clover, red clover and Italian ryegrass, Italian ryegrass. The highest spring barley yield characterized by the best biometrical characteristics of the ear and 1000-grain weight was recorded in the treatment with *Azotobacter chroococcum, Pseudomonas fluorescens*, *Bacillus subtilis*, and *Bacillus amyloliquefaciens*. Of the tested living mulch, the most favorable effect on grain yield and its structure was associated with the living mulch of red clover mixed with Italian ryegrass. Organic growers should be encouraged to apply the technology of cultivating spring barley with a mixture of red clover and Italian ryegrass used as living mulch following an application of *Azotobacter chroococcum, Pseudomonas fluorescens*, *Bacillus subtilis*, and *Bacillus amyloliquefaciens*, as it contributes to the highest grain yield with good yield structure while preserving the highest soil fertility.

Keywords: spring barley, plant growth promoting rhizobacteria, living mulch, yield, biological index of soil fertility

1. INTRODUCTION

Cereals are the largest product group in the world in terms of cultivated area and production volume. Thus, cereals are sown on nearly half of the world's arable land (Çağlar and Bulut, 2023). According to Hlisnikovský *et al*. (2023), yields from cereal crops depend mostly on the supply of nutrients to the plants and weather conditions. Due to the increase in the world population and the associated need to increase food production, the use of mineral fertilizers is increasing worldwide (Zhang and Zhang, 2007). However, the global efficiency of mineral fertilizer use is low, amounting to *e.g.* only 35% for nitrogen mineral fertilizers (Omara *et al*., 2019). This leads to a significant environmental burden (Spiertz, 2009; Ghimire *et al*., 2023) as well as an increase in the cost intensity of grain cultivation (Herrera *et al*., 2016). In addition, the introduction of the European Commission European Green Deal implies, among other things, a reduction in the use of mineral fertilizers and the development of organic farming to make Europe a climate-neutral continent by 2050 (European Commission, 2019). In view of these premises, it is reasonable to look for alternative solutions to provide plant nutrients and to develop organic farming without negatively affecting yields obtained from crops as well as their quality. A promising alternative to conventional mineral

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*Corresponding author e-mail: rafal.gorski@pansim.edu.pl fertilization that can be used in organic farming is, increasingly researched worldwide, the use of Plant Growth Promoting Rhizobacteria (PGPR) (Reed and Glick, 2023). The great interest in PGPR in agriculture is primarily due to their positive effects on plant growth and development by increasing plant biomass, plant mineral content, and root length and by ensuring protection against pathogens and increasing tolerance to various abiotic stresses (Reed and Glick, 2023). Counteracting or mitigating the effects of abiotic stresses is particularly important due to the ongoing climate change, which is resulting in increasingly frequent periods with precipitation deficits and high air temperatures (Koryagin *et al*., 2022). The direct effects of PGPR on crops have been attributed to a number of mechanisms, which can vary from one bacterial strain to another (Glick, 1995). The most commonly reported direct mechanisms of PGPR in the literature include facilitating mineral dissolution and absorption (Hashem *et al*., 2019), biological nitrogen fixation (BNF) (Ladha *et al*., 2022), synthesis of phytohormones such as cytokinin, gibberellin, and auxin (Minuț *et al*., 2023), and modulation of plant ethylene and 1-aminocyclopropane-1-carboxylate (ACC) levels through the enzyme ACC deaminase (Sati *et al*., 2023). The positive effect of PGPR is also seen in improving soil fertility (El-Shamy *et al*., 2022). However, the efficacy of PGPR application in crop cultivation may be influenced by several factors, including bacterial species and strain, crop management, and plant genotype (Tahir *et al*., 2015). Thus, there are doubts in the literature about the effectiveness of bacterial formulations in varying climate and soil conditions (Herrera *et al*., 2016). Therefore, it is reasonable to test different bacteria in given climatic conditions as well as in different agricultural systems in order to determine the most effective measures to implement in agricultural practice.

To conserve natural resources, agroecosystems in agriculture should be diversified, which can be achieved by increasing the number of plant species grown (Wittwer *et al*., 2017). An increase in biodiversity can be achieved by using living mulches (LM) in the main crop. Cultivation with LM, in addition to increasing the biodiversity of agricultural areas, can also bring a number of other benefits. In ongoing field studies in various areas around the world, researchers have found improved soil quality (Poeplau and Don, 2015), minimized leaching of nutrients from the soil (Poudel *et al*., 2022), and improved soil water conditions (Boyd *et al*., 2001). A very important benefit of growing cereals with LM, especially in organic farming systems, is also the reduction of weeds (Verret *et al*., 2017; Płaza *et al*., 2023). The indicated benefits of growing cereals with LM can lead to an increase in the yield of the main crop (Løes *et al*., 2011); however, in the available literature, the data on this subject are inconclusive, as there are publications reporting a reduction in the yield obtained as a result of growing with LM (Bhaskar *et al*., 2014). The discrepant data on the effect of LM on main crops may be due to the type of crops constituting LM as well as the timing of their sowing in relation to the main crop (Afshar *et al*., 2018).

Analyzing the benefits that the use of PGPR and cultivation with LM can bring to agriculture, an attempt was made to combine these crop management techniques. The aim of the field research conducted was to evaluate the yield and yield structure of spring barley and the biological soil fertility index (BIF) as a result of the application of bacterial consortia and cultivation with LM. The research hypothesis assumed that the applied factors of the experiment would allow the development of an organic cultivation technology for spring barley based on the selection of the optimal variant consisting of constructed bacterial consortia and LM providing the highest grain yield, the best possible yield structure, and the highest level of BIF.

2. MATERIALS AND METHODS

2.1. Experimental design

The field research was conducted in central Europe, Poland, in a temperate climate from 2019 to 2021 on an organic farm (52°12′35″N 22°11′05″E). The field experiment was implemented on Stagnic Luvisol soil. The soil conditions before the experiment was established are shown in Table 1. The weather conditions during the implementation of the field research are shown in Fig. 1.

Two factors were analyzed in the field experiment: A – the use of constructed bacterial consortia: control (no bacterial consortia), bacterial consortium 1 – *Azospirillum lipoferum* Br17 and *Azotobacter chroococcum*, bacterial consortium 2 – *Pseudomonas fluorescens*, *Bacillus subtilis*, and *Bacillus amyloliquefaciens*, and bacterial consortium 3 – *Azotobacter chroococcum, Pseudomonas fluorescens*,

Table 1. Soil conditions when conducting field research

Levels of available mineral nutrients (mg kg^{-1} soil)				pH in KCL	Organic carbon (g kg^{-1} soil)
P	K	Mg	N		
83	121	42	8.9	6.1	10.5
Granulometric composition of the soil (%)					
2.0-0.05 mm		0.05-0.02 mm	0.02-0.002 mm		<0.002 mm
79.49		9.58	9.57		1.37

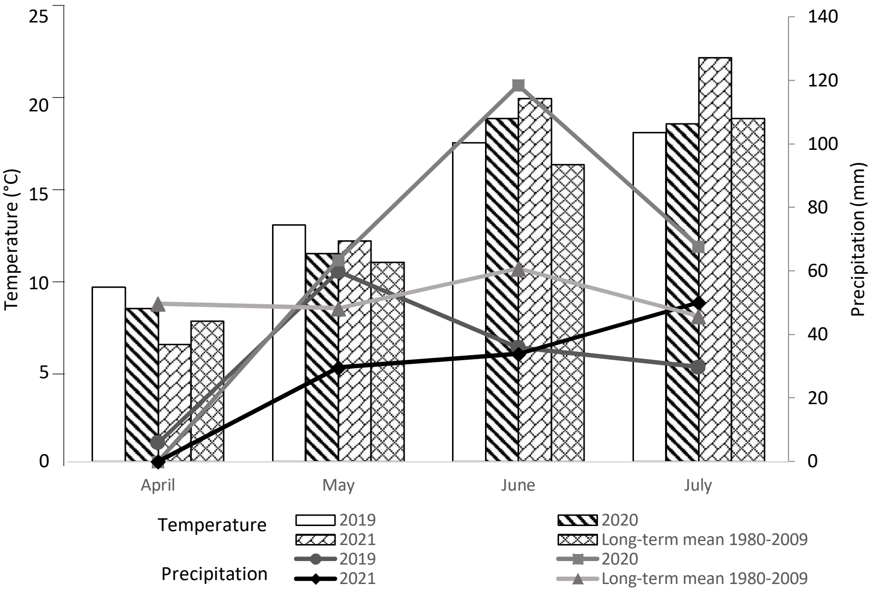

Fig. 1. Distribution of temperatures and precipitation during spring barley growing seasons according to the Zawady Meteorological Station.

Bacillus subtilis, and *Bacillus amyloliquefaciens*; B – cultivation with living mulch: control (no LM), red clover, red clover + Italian ryegrass, Italian ryegrass. The experiment was conducted in a split-block design with three replicates in each of the three years of the research. The total number of experimental objects in the 1st year of the research was 48. The area of one experimental object was 20 m^2 (5 × 4 m). The crop preceding the spring barley was winter rye. After harvesting the winter rye, post-harvest cultivation was carried out. In October (autumn), goat manure was applied at a rate of 15 t ha^{-1}. In early April (spring), the spring barley with the LM was sown on the same day. The seeding rates of spring barley, red clover, the red clover plus Italian ryegrass mixture, and Italian ryegrass were 160, 16, 9 + 15, and 30 kg ha^{-1}, respectively. The spring barley was sown with the use of a grain drill with a 12.5 cm row spacing and a depth of 5-6 cm. The LM was then sown in the barley rows, 1-2 cm deep with a row spacing of 12.5 cm. Bacteria *Azospirillum lipoferum* Br 17 were applied twice during the growing season. Firstly, the barley grain was treated with an inoculant suspension (100 mL 15 kg^{-1} grain) followed by inoculant spraying (the inoculant rate of 1 L/150 L water ha^{-1}) at the emergence stage (BBCH 10-15). Bacteria *Azotobacter chroococcum* were applied as two inoculant spraying applications performed during the growing season (the first application time '0' on the sowing day, the second application - the BBCH 29-30 scale). The inoculant rate was 1 L/250 L water ha^{-1}. Additionally, the application of the bacterial consortium of *Pseudomonas fluorescens*, *Bacillus subtilis*, and *Bacillus amyloliquefaciens* and the bacterial consortium of *Azotobacter chroococcum, Pseudomonas fluorescens*, *Bacillus subtilis* and *Bacillus amyloliquefaciens* was conducted twice during the growing season (the first application time '0' on the sowing day and the second application – the BBCH 29-30 scale). The following rate of the inoculant was applied: 1 L/250 L water ha^{-1}.

2.2. Bacterial consortia

The bacterial species used for inoculation were sourced from the Department of Soil Science and Microbiology at the University of Life Sciences in Poznań, Poland. The metabolic properties of the bacterial species used in the field experiment and the selection process to construct the applied proprietary microbial consortia in the experiment were detailed by Płaza *et al.* (2022) and Niewiadomska *et al.* (2023).

2.3. Data collection

Spring barley was harvested in late July (summer) from a 1 m^2 plot using electric shears. Immediately before the harvest, 10 ears from each plot were sampled to determine ear length, number of grains per ear, and grain weight per ear. During harvesting, the grain yield was determined in each plot and converted to tons per hectare. Then, samples were also taken to determine the weight of 1000 grains. During the spring barley growing season, soil samples were collected to assess the biological index of soil fertility (BIF). The soil samples were taken on three dates: date I (BIF I) at the stage of spring barley emergence (BBCH 16-17), date II (BIF II) at the stage of spring barley flowering (BBCH 61-65), and date III (BIF III) post-harvest. The BIF (biological index of soil fertility) was calculated based on DHA (dehydrogenase activity) determined using the colorimetric method (Thalmann, 1968), with 1% TTC (triphenyl tetrazolium chloride) as a substrate, after 24 h incubation at 30°C, at a wavelength of 485 nm, expressed as µmol TPF g^{-1} dm soil 24 h^{-1} and CAT (catalase activity) determined by permanganometry according to Johnson and Temple (1964) with 0.3% H_2O_2 as a substrate, after 20 min incubation at room temperature (approx. 20°C), titration with 0.02 M $KMnO_4$ to a light pink color, expressed as µmol H_2O_2 g^{-1} dm soil min^{-1}, using the formula (DHA + kCAT)/2, where *k* is the coefficient of proportionality (0.01) according to the method described by Stefanic *et al.* (1984).

2.4. Statistical Analysis

Statistical analysis was performed using tree-way analysis of variance (ANOVA) using the software package Statistica version 13.3. The significance of the sources of variation was tested using the Fisher-Snedecor F test ($F \leq 0.05$). Tukey's honestly significant difference test (HSD post-hoc test) was used to determine the differences among means ($p<0.05$). Pearson's correlation coefficient was calculated to evaluate the strength of the linear association between the variables studied.

3. RESULTS

3.1. Grain yield

The field research demonstrated a significant effect of the use of the bacterial consortia ($p<0.001$) and living mulches ($p<0.001$) and an interaction of LM x bacterial consortia ($p<0.05$) on the spring barley grain yield (Table 2).

The lowest spring barley grain yield was obtained on sites where no bacterial consortium was applied. The application of consortium 1, containing bacteria *Azospirillum lipoferum* Br17 and *Azotobacter chroococcum*, and consortium 2, which included bacteria *Pseudomonas fluorescens*, *Bacillus subtilis*, and *Bacillus amyloliquefaciens*, resulted in an increase in the obtained yield by 25% on average. No statistically significant differences were found between the objects on which bacterial consortia 1 and 2 were applied. The significantly highest yield of spring barley was found in the experimental treatment where bacterial consortium 3, containing bacteria *Azotobacter chroococcum, Pseudomonas fluorescens*, *Bacillus subtilis*, and *Bacillus amyloliquefaciens*, was applied. An average increase of 45% in the spring barley grain yield was revealed in relation to the control objects. Also, the cultivation of spring barley with the LM had an impact on the obtained final grain yield (Table 2). The lowest yields were obtained on the objects where no LM was applied. The application of the LM of Italian ryegrass resulted in a 4% increase in the grain yield but this difference from the control object was not significant. On the other hand, significantly higher spring barley yields were obtained with the application of the LM of red clover (22% increase), and the LM of a mixture of red clover + Italian ryegrass contributed to the significantly highest yields (35% increase). The field tests demonstrated an interaction of the experimental factors (Table 2). The lowest yields of spring barley were demonstrated on the objects where no LM was applied and on the objects with the LM of a mixture of red clover + Italian ryegrass and without the application of the bacterial consortia. The application of bacterial consortia 1 and 2 resulted in a significant increase in the grain yield. No significant differences were found between the indicated variants. In contrast, the highest grain yields were obtained after the application of bacterial consortium 3. On objects with the LM of red clover, the lowest yields were noted without the application of any constructed bacterial consortium; they were higher after the application of consortium 1, consecutively after the use of consortium 2, and significantly highest on objects where bacterial consortium 3 was applied. Also on objects with the LM of Italian ryegrass, the highest grain yields were found after the use of bacterial consortium 3; in addition, no significant difference was found between these objects and those on which consortium 1 was applied. Similarly, there were no significant differences in grain yields after the application of consortia 1 and 2.

Table 2. Spring barley grain yield according to bacterial consortia and living mulch (t ha^{-1}) (means across 2019-2021)

Bacterial consortia (A)	Living mulch (B)				
	Control	Red clover	Red clover + Italian ryegrass	Italian ryegrass	Means
Control	3.08 ± 0.64 c	3.75 ± 0.49 d	3.95 ± 0.62 c	3.23 ± 0.71 c	3.50 ± 0.72 C
1	3.82 ± 0.74 b	4.34 ± 0.58 c	5.33 ± 1.03 b	3.98 ± 0.82 ab	4.37 ± 1.00 B
2	3.80 ± 0.44 b	4.89 ± 0.84 b	4.90 ± 0.61 b	3.95 ± 0.51 b	4.39 ± 0.80 B
3	4.31 ± 0.55 a	5.37 ± 0.63 a	6.15 ± 1.11 a	4.44 ± 0.60 a	5.07 ± 1.06 A
Means	3.75 ± 0.75 C	4.59 ± 0.89 B	5.08 + 1.18 A	3.90 ± 0.8 C	
P values	A: <0.001, B <0.001, B×A <0.05				

1 – *Azospirillum lipoferum* Br17*, Azotobacter chroococcum*; 2 – *Pseudomonas fluorescens*, *Bacillus subtilis*, *Bacillus amyloliquefaciens*; 3 – *Azotobacter chroococcum, Pseudomonas fluorescens*, *Bacillus subtilis* and *Bacillus amyloliquefaciens*, ± standard deviation.

Table 3. Spring barley grain yield according to bacterial consortia (t ha^{-1})

Bacterial consortia (A)	Years (Y)		
	2019	2020	2021
Control	2.88 ± 0.57 d	4.30 ± 0.38 c	3.33 ± 0.16 c
1	3.27 ± 0.49 c	5.08 ± 0.77 b	4.76 ± 0.54 b
2	3.59 ± 0.37 b	4.98 ± 0.68 b	4.60 ± 0.55 b
3	4.16 ± 0.59 a	5.86 ± 1.12 a	5.19 ± 0.55 a
Means	3.48 ± 0.69 C	5.05 ± 0.96 A	4.47 ± 0.84 B
P values	Y: <0.01, Y×A: <0.01		

Explanations as in Table 2.

Table 4. Spring barley grain yield according to living mulch (t ha^{-1})

Living mulch (B)	Years (Y)		
	2019	2020	2021
Control	2.95 ± 0.49 c	4.33 ± 0.37 d	3.98 ± 0.51 d
Red clover	3.85 ± 0.46 b	5.34 ± 0.70 b	4.57 ± 0.75 b
Red clover + Italian ryegrass	4.09 ± 0.52 a	6.02 ± 1.06 a	5.14 ± 0.93 a
Italian ryegrass	3.01 ± 0.46 c	4.51 ± 0.28 c	4.18 ± 0.61 c
P values	Y × B: <0.01		

± standard deviation.

The growing season conditions also had a significant influence (p<0.01) on the spring barley grain yield (Table 3). The lowest grain yield was obtained in 2019, which was characterized by the lowest precipitation. A 28% higher yield of spring barley was obtained in 2021. Significantly, the highest yield was registered in 2020, *i.e.* the year with the highest precipitation. With respect to 2019, the grain yield in 2020 was higher by nearly 45%. The study revealed a significant interaction in the years of the experiment and the bacterial consortia used (p<0.01). In 2020 and 2021, significantly the highest grain yield was achieved after the use of bacterial consortium 3, significantly lower with consortium 1, and consecutively after consortium 2. However, on all sites, the use of the bacterial consortia resulted in higher yields, compared to the control objects. In 2019, the highest yield was also obtained after the use of bacterial consortium 3. However, in contrast to the previously discussed growing seasons, in the conditions with the lowest total precipitation, higher yields were obtained after applying bacterial consortium 2 than 1. Similarly to the other years of the research, the lowest yields in 2019 were obtained on objects where no bacterial consortia were applied.

The field experiment also revealed a statistically significant interaction (p<0.01) of the years of the research x LM (Table 4). In 2019, the lowest spring barley grain yield was obtained on the objects with the LM of Italian ryegrass and on the objects without LM. A higher yield was noted in the variant with the LM of red clover, while the highest yield was achieved with the LM of a mixture of red clover + Italian ryegrass. In 2020 and 2021, the highest grain yields were registered on objects where the LM was a mixture of red clover + Italian ryegrass, but they were lower when the LM of red clover was used. The lowest yields among the LM used were obtained with the use of Italian ryegrass. However, in all cases of spring barley cultivation with LM, higher grain yields were noted, in comparison to the cultivation without LM.

3.2. Ear length

The field research demonstrated significant effects of the applied bacterial consortia (p<0.001), LM (p<0.001) and an interaction of LM x bacterial consortia (p<0.01) on the spring barley ear length (Table 5).

The shortest ears were obtained on objects where no microbial preparations were applied. The use of bacterial consortium 2 in the crop increased the average ear length by 16%, while bacterial consortium 1 increased it by 21%. The longest spring barley ears were registered after the use of bacterial consortium 3. In comparison to the objects without the bacterial consortium, the use of bacterial consortium 3 increased the ear length by 43%. The cultivation

Table 5. Spring barley ear length according to bacterial consortia and living mulch (cm) (means across 2019-2021)

Bacterial consortia (A)	Living mulch (B)				
	Control	Red clover	Red clover + Italian ryegrass	Italian ryegrass	Means
Control	5.2 ± 1.0 d	5.9 ± 1.2 d	5.7 ± 1.2 d	5.4 ± 1.3 c	5.6 ± 1.4 D
1	6.4 ± 1.1 b	6.9 ± 1.2 b	7.2 ± 1.2 b	6.6 ± 1.4 b	6.8 ± 1.6 B
2	6.2 ± 1.1 c	6.6 ± 1.2 c	6.9 ± 1.0 c	6.4 ± 1.4 b	6.5 ± 1.5 C
3	7.4 ± 1.2 a	8.7 ± 1.3 a	8.2 ± 1.2 a	7.6 ± 1.3 a	8.0 ± 1.4 A
Means	6.3 ± 1.1 B	7.0 ± 1.2 A	7.0 ± 1.2 A	6.5 ± 1.4 B	
P values	A: <0.001, B <0.001, B×A <0.01				

Explanations as in Table 2.

Table 6. Spring barley grain number per ear according to bacterial consortia and living mulch (pcs) (means across 2019-2021)

Bacterial consortia (A)	Living mulch (B)				
	Control	Red clover	Red clover + Italian ryegrass	Italian ryegrass	Means
Control	15 ± 3 d	20 ± 3 d	18 ± 3 d	16 ± 2 d	17 ± 5 D
1	22 ± 3 b	24 ± 3 b	26 ± 3 b	23 ± 3 b	24 ± 4 B
2	20 ± 3 c	22 ± 2 c	24 ± 2 c	21 ± 3 c	22 ± 5 C
3	26 ± 2 a	28 ± 2 a	30 ± 3 a	27 ± 2 a	28 ± 5 A
Means	21 ± 3 D	23 ± 3 B	25 ± 3 A	22 ± 3 C	
P values	A: <0.001; B <0.01; B×A <0.01				

Explanations as in Table 2.

of spring barley with the LM of Italian ryegrass resulted in an increase in ear length by only 3%. No statistically significant differences were found between the control objects without LM and with the LM of Italian ryegrass. In contrast, growing spring barley with the LM of red clover and the mixture of red clover + Italian ryegrass resulted in an 11% increase in ear length, compared to the control objects. The interaction of LM x bacterial consortia demonstrated that, regardless of the presence of absence of LM, the highest spring barley ear length was noted on the objects where bacterial consortium 3 was applied, while it was lower when the other bacterial consortia were applied. Significantly, the smallest ear length was found on the objects without the application of the bacterial consortia, regardless of the LM experimental factor.

3.3. Number of grains per ear

The application of the bacterial consortia ($p<0.001$), the cultivation of spring barley with LM ($p<0.01$), and the interaction of LM x bacterial consortia ($p<0.01$) significantly influenced the number of grains per spring barley ear (Table 6).

The smallest number of grains per ear was found in the control objects where the constructed bacterial consortia were not applied, while a significantly higher number of grains per ear was found after their application. The use of bacterial consortium 2 resulted in a 29% increase, while consortium 1 resulted in a 41% increase. The highest number of grains in an ear of spring barley was found after inoculation with bacterial consortium 3, where an increase of 65% was proven in relation to the control objects. Also, the cultivation of spring barley with LM caused an increase in the number of grains per ear. The cultivation of spring barley with the LM of Italian ryegrass resulted in an increase in the number of grains per ear by 5%, while the LM of red clover increased this parameter by 10%. The largest increase in the number of grains per ear (an increase by 19%) was obtained when spring barley was grown with the LM of a mixture of red clover + Italian ryegrass. The revealed interaction of LM x bacterial consortia demonstrated that, regardless of the presence of LM or cultivation without LM, the highest number of grains per ear was recorded in the objects where bacterial consortium 3 was used. A lower number was recorded on the objects with the other bacterial consortia and the significantly lowest number was obtained when no bacterial consortia were used.

3.4. Grain weight per ear

Grain weight per ear was significantly differentiated by the use of the bacterial consortia ($p<0.01$), cultivation with LM ($p<0.05$), and the interaction of these factors of the field experiment ($p<0.05$) (Table 7).

The smallest grain weight per ear of spring barley was recorded in the control objects where no constructed bacterial consortia were applied. The application of bacterial consortia 1 and 2 resulted in a significant increase in grain weight per ear, by 52 and 46%, respectively, compared to the control objects. On the other hand, the significantly highest grain weight per ear was revealed after the application of bacterial consortium 3. On these objects, an average increase of 121% in grain weight was obtained, compared to objects where spring barley was grown without the application of the engineered bacterial inoculants. The application of the LM of Italian ryegrass and the cultivation of spring barley without LM demonstrated significantly the lowest grain weight per ear. The average difference between the objects was only about 3%. A 20% higher grain weight of was noted when spring barley was grown with the LM of red clover. In contrast, spring barley grown with the LM of a mixture of red clover + Italian ryegrass showed significantly the highest grain weight per ear. The revealed interaction demonstrated that, in all the objects with LM and the control objects, the highest grain weight per ear was demonstrated by spring barley grown with the use of bacterial consortium 3. Lower values were obtained when consortia 1 and 2 were used. In all the experimental objects, the use of the bacterial consortia increased grain weight per ear, compared to the objects without their application.

Table 7. Spring barley grain weight per ear according to bacterial consortia and living mulch (g) (means across 2019-2021)

Bacterial consortia (A)	Living mulch (B)				
	Control	Red clover	Red clover + Italian ryegrass	Italian ryegrass	Means
Control	0.42 ± 0.12 c	0.64 ± 0.11 c	0.56 ± 0.10 c	0.43 ± 0.12c	0.51 ± 0.23 C
1	0.73 ± 0.10 b	0.79 ± 0.11 b	0.84 ± 0.10 b	0.75 ± 0.10 b	0.78 ± 0.25 B
2	0.70 ± 0.11 b	0.74 ± 0.10 b	0.81 ± 0.11 b	0.73 ± 0.10 b	0.74 ± 0.30 B
3	0.98 ± 0.10 a	1.22 ± 0.10 a	1.32 ± 0.11 a	1.00 ± 0.12 a	1.13 ± 0.23 A
Means	0.71 ± 0.14 C	0.85 ± 0.12 B	0.88 ± 0.12 A	0.73 ± 0.18 C	
P values	A: <0.01; B <0.05; B×A <0.05				

Explanations as in Table 2.

Table 8. Spring barley 1 000 grain yield according to bacterial consortia and living mulch (g) (means across 2019-2021)

Bacterial consortia (A)	Living mulch (B)				
	Control	Red clover	Red clover + Italian ryegrass	Italian ryegrass	Means
control	28.7 ± 5.6 c	30.6 ± 5.0 d	29.5 ± 5.6 d	29.0 ± 5.0 c	29.5 ± 5.8 C
1	31.4 ± 5.0 b	32.7 ± 5.6 b	33.6 ± 5.4 b	31.7 ± 5.1 b	32.4 ± 5.9 B
2	30.6 ± 5.0 c	31.5 ± 5.6 c	32.7 ± 5.6 c	31.0 ± 5.4 b	31.4 ± 6.6 B
3	35.2 ± 5.6 a	37.4 ± 5.0 a	39.8 ± 5.0 a	35.8 ± 5.2 a	37.0 ± 5.9 A
Means	31.5 ± 5.4 C	33.0 ± 5.4 B	33.9 ± 5.5 A	31.9 ± 5.6 C	
P values	A: <0.001; B <0.001; B×A <0.01				

Explanations as in Table 2.

3.5. Weight of 1000 grains

The weight of 1000 grains of spring barley was significantly influenced by the use of the constructed proprietary bacterial consortia (p<0.001), LM (p<0.001) and the interaction of LM x bacterial consortia (p<0.01) (Table 8).

The smallest weight of 1000 grains of spring barley was revealed on the control objects without the use of the constructed bacterial consortia. The use of consortia 1 and 2 increased the weight of 1000 grains by 10 and 7%, respectively, compared to the control objects. No additional statistically significant differences were found between these consortia. The highest weight of 1000 grains was obtained on the objects where bacterial consortium 3 was applied. Compared to the control objects, the increase in the weight of 1 000 grains in this case was 26%. The cultivation of spring barley with LM also affected the weight of 1 000 grains. The lowest weight of 1 000 grains was recorded when spring barley was grown without LM and when Italian ryegrass was used as the LM. The difference between these objects was less than 1%. A higher weight of 1 000 grains was registered on the objects where spring barley was grown with the LM of red clover and the mixture of red clover + Italian ryegrass. Compared to the control objects without LM, a 5% increase in the weight of 1000 grains was achieved upon the application of the LM of red clover and 8% when the LM of a mixture of red clover + Italian ryegrass was used. The field study demonstrated an interaction showing that, when spring barley was grown without LM and with the LM of red clover and the mixture of red clover + Italian ryegrass, the lowest 1000-grain weight was recorded on sites without the use of the constructed proprietary bacterial inoculants. Successively, the application of the particular bacterial consortia caused an increase in the weight of 1000 grains on these objects. The highest weight of 1000 grains was observed after the application of bacterial consortium 3. In the objects where Italian ryegrass was used as the LM, the lowest weight of 1000 grains was registered in the absence of the bacterial consortia, while the highest values were noted after the use of bacterial consortium 3. In addition, there were no significant differences in the weight of 1 000 grains of spring barley after the application of consortia 1 and 2.

Table 9. Biological index of soil fertility BIF I determined after spring barley emergence (means across 2019-2021)

Bacterial consortia (A)	Living mulch (B)				
	Control	Red clover	Red clover + Italian ryegrass	Italian ryegrass	Means
Control	1.46 ± 0.34 b	1.79 ± 0.76 c	2.14 ± 0.80 c	1.53 ± 0.67 b	1.73 ± 0.87 C
1	1.99 ± 0.34 ab	2.42 ± 0.76 b	3.53 ± 0.82 a	2.05 ± 0.62 ab	2.50 ± 0.75 B
2	1.92 ± 0.38 ab	3.17 ± 0.92 a	2.82 ± 0.75 b	1.99 ± 0.81 ab	2.48 ± 1.24 B
3	2.45 ± 0.51 a	3.40 ± 0.69 a	4.09 ± 0.71 a	2.52 ± 0.51 a	3.12 ± 0.21 A
Means	1.96 ± 0.53 C	2.69 ± 0.83 B	3.15 ± 0.95 A	2.02 ± 0.95 C	
P values	A: <0.001; B <0.001; B×A <0.001				

Explanations as in Table 2.

3.6. BIF I

The BIF value at the spring barley emergence stage was significantly influenced by the use of the constructed bacterial consortia (p<0.001) and cultivation with LM (p<0.001). A statistically significant interaction of LM x bacterial consortia was also revealed (p<0.001) (Table 9).

The lowest BIF I value was revealed in the control objects where no bacterial consortia were applied. It was significantly higher after the use of consortia 1 and 2. The value of BIF I was 44% and 43% higher in the control objects, respectively, after the application of consortia 1 and 2. Significantly, the highest value of BIF I was revealed in the objects where bacterial consortium 3 was applied, where a value higher by 80% was obtained in relation to the control objects. Also, the cultivation with LM had an effect on the BIF value at the barley emergence stage. The lowest value was revealed on the objects where no LM was sown and when Italian ryegrass was used as LM. The difference between these objects was 3%. A higher value of BIF I was registered when spring barley was grown with the LM of red clover. Compared to the control objects, the increase in BIF I was 38%. The highest BIF I value was revealed on the objects where spring barley was grown with the LM of a mixture of red clover + Italian ryegrass. The difference between these objects and the barley crop without LM was 61%. The interaction of LM x bacterial consortia revealed in the field experiment demonstrated that, in the absence of LM and when Italian ryegrass was used as LM, the highest BIF I value was noted in the variant with bacterial consortium 3. However, the value obtained was not significantly different from BIF I obtained after using consortia 1 and 2. The lowest BIF I on these objects, on the other hand, was revealed when no bacterial consortia were applied. However, this value was also not statistically different from BIF I after the application of consortia 1 and 2. On the experimental sites where spring barley was grown with the LM of red clover, the highest value of BIF I was revealed after the use of constructed bacterial consortia 2 and 3, lower after the use of consortium 1, and the lowest in the absence of the bacterial consortium. On the other hand, when spring barley was grown with the LM of a mixture of red clover + Italian ryegrass, the highest BIF I was demonstrated after the use of bacterial consortia 1 and 3, while it was lower when consortium 2 was applied and the lowest when the constructed bacterial inoculants were not applied.

3.7. BIF II

BIF at the flowering stage of spring barley was significantly differentiated by the application of the composed bacterial consortia (p<0.001) (Table 10).

The lowest value of BIF II was found on objects where no bacterial consortia were applied. There was a significant increase in BIF II after the application of the constructed bacterial consortia 1 and 2, by 52 and 47% respectively, compared to the control objects. On the other hand, the highest value of BIF II was demonstrated on the objects where bacterial consortium 3 was applied. Compared to the control objects, the use of bacterial consortium 3 resulted in an 82% increase in BIF II. The value of BIF II was also influenced by the application of LM in the spring barley (p<0.001) (Table 10). The lowest BIF II was obtained when spring barley was grown without LM and on objects where Italian ryegrass was used as LM. The application of the LM of red clover caused a 17% increase in the BIF II values, while the LM of a mixture of red clover + Italian ryegrass increased by 31%. The experiment demonstrated an interaction of LM x bacterial consortia (p<0.001). On the objects where spring barley was grown without LM and with the Italian ryegrass LM, the highest value of BIF II was registered after the application of bacterial consortium 3, while the lowest value was obtained in the absence of the bacterial consortia. In addition, no significant difference between the application of bacterial consortia 1 and 2 was found in these objects. When spring barley was grown with the LM of red clover, the highest BIF II value was recorded when using the constructed bacterial consortia 2 and 3, lower when using consortium 1, and the lowest in the objects where no constructed bacterial consortia were

Table 10. Biological index of soil fertility BIF II determined at the stage of spring barley flowering (means across 2019-2021)

Bacterial consortia (A)	Living mulch (B)				
	Control	Red clover	Red clover + Italian ryegrass	Italian ryegrass	Means
control	2.84 ± 1.00 c	3.20 ± 1.22 c	3.60 ± 0.79 d	2.90 ± 1.15 c	3.14 ± 1.12 C
1	4.28 ± 1.04 b	4.78 ± 1.13 b	5.70 ±1.23 b	4.32 ± 1.35 b	4.77 ± 1.53 B
2	4.10 ± 1.25 b	5.31 ± 1.55 a	5.10 ± 0.89 c	4.01 ± 1.15 b	4.63 ± 1.49 B
3	5.05 ± 1.00 a	5.74 ± 1.26 a	6.97 ± 0.99 a	5.09 ± 1.19 a	5.72 ± 1.36 A
Means	4.07 ± 1.12 C	4.76 ± 1.42 B	5.34 ± 1.14 A	4.08 ± 1.59 C	
P values	A: <0.001; B <0.001; B×A <0.001				

Explanations as in Table 2.

Table 11. Biological index of soil fertility BIF III determined after spring barley harvest (means across 2019-2021)

Bacterial consortia (A)	Living mulch (B)				
	Control	Red clover	Red clover + Italian ryegrass	Italian ryegrass	Means
control	0.73 ± 0.32 b	1.44 ± 0.58 c	1.83 ± 0.74 c	1.14 ± 0.68 c	1.28 ± 0.73 C
1	1.26 ± 0.49 ab	2.55 ± 0.84 b	3.11 ± 0.87 b	2.10 ± 0.84 b	2.25 ± 1.42 B
2	1.17 ± 0.71 ab	2.69 ± 0.74 b	3.25 ± 0.92 b	2.06 ± 0.78 b	2.29 ± 1.52 B
3	1.52 ± 0.82 a	3.29 ± 1.19 a	4.98 ± 0.90 a	2.74 ± 1.08 a	3.13 ± 1.18 A
Means	1.17 ± 0.96 D	2.49 ± 1.31 B	3.29 ± 1.54 A	2.01 ± 1.29 C	
P values	A: <0.001; B <0.001; B×A <0.001				

Explanations as in Table 2.

used. On the other hand, in the objects where spring barley was grown with the LM of a mixture of red clover + Italian ryegrass, the highest value of BIF II was demonstrated after the application of bacterial consortium 3, and a lower value was recorded when bacterial consortia 1 and 2 were applied consecutively. The lowest value on these experimental objects was demonstrated in the variant with no application of the bacterial consortia.

3.8. BIF III

The post-harvest BIF value of spring barley was significantly influenced by the use of the constructed bacterial consortia (p<0.001) (Table 11).

The lowest BIF III value was obtained in the objects where no bacterial consortia were used. A significantly higher value, not significantly different between each other, was obtained after the use of consortia 1 and 2. Compared to the BIF III values in the control objects, the application of bacterial consortium 1 resulted in a 76% increase, while consortium 2 resulted in a 79% increase. The highest post-harvest BIF value of spring barley was demonstrated in the objects where bacterial consortium 3 was applied. The increase in the BIF value in these objects, compared to the control objects, was 145%. BIF III was also significantly different as a result of the cultivation of spring barley with LM (p<0.001) (Table 11). The lowest post-harvest BIF was obtained in the objects where spring barley was grown without LM. The cultivation of spring barley with the LM of Italian ryegrass resulted in a 72% increase in BIF III, while the LM of red clover induced a 113% increase in BIF III, compared to the objects without LM. The highest BIF III value was revealed when the LM of a mixture of red clover + Italian ryegrass was used. The noted BIF value in this case was 181% higher than that obtained in the control objects. The field experiment also revealed an interaction of LM x bacterial consortia (p<0.001) (Table 11). In the objects where spring barley was grown with LM, the highest value of BIF III was recorded after the use of bacterial consortium 3, lower after the application of consortia 1 and 2, and the lowest in the absence of the bacterial consortia. On the other hand, in the case of spring barley cultivation without LM, the highest value was also registered after the use of bacterial consortium 3; in addition, no significant differences were found between all the constructed consortia applied. The lowest value of BIF III was found in the objects where the bacterial consortia were not applied;

Table 12. Correlation coefficients (n = 144) between spring barley grain yield, yield structure and BIF

	Grain yield	Grain number per ear	Grain weight per ear	Ear length	1 000 grain weight
Grain number per ear	0.8499 **	-	-	-	-
Grain weight per ear	0.8346 **	0.9241 **	-	-	-
Ear length	0.8376 **	0.8463 **	0.8386 **	-	-
1 000 grain weight	0.8201 **	0.8230 **	0.7659 **	0.8037 **	-
BIF I	0.6245 **	0.5001 **	0.5000 **	0.7968 **	0.4084 **
BIF II	0.9434 **	0.8835 **	0.8420 **	0.9069 **	0.8693 **
BIF III	0.8801 **	0.7529 **	0.7440 **	0.8790 **	0.7015 **
Significance: $p \leq 0.01$**					

however, there were also no statistically significant differences between these objects and the objects where bacterial consortia 1 and 2 were applied.

3.9. Correlation

Pearson correlation analysis demonstrated a highly significant relationship between the analyzed variables (Table 12). A highly significant positive correlation was found between the grain yield and the number and weight of grains per spike, spike length, and 1 000-grain weight. The yield structure parameters were additionally highly correlated among themselves. The correlation coefficients further demonstrated highly significant correlations between the spring barley grain yield and yield structure parameters and the BIF determined after plant emergence (BIF I), at the stage of flowering (BIF II), and after harvest of spring barley plants (BIF III).

4. DISCUSSION

4.1. Grain yield and yield structure of spring barley

Soil microbial activity significantly influences plant growth and development (Rashid *et al*., 2016). Therefore, by modifying the useful bacteria of the rhizosphere, crop yields can be significantly increased (Shalaby *et al*., 2023). This statement is confirmed by the results of the present research. The use of a composed bacterial consortium containing bacteria *Azospirillum lipoferum* Br17 and *Azotobacter chroococum* as well as a consortium containing bacteria *Pseudomonas fluorescens*, *Bacillus subtilis*, and *Bacillus amyloliquefaciens* resulted in a comparable increase in the obtained yield of spring barley, weight of grains per ear, and the weight of 1000 grains. Also, Gaspareto *et al*. (2023) demonstrated an increase in wheat yields after the application of *Azospirillum brasilense* and *Bacillus subtilis* bacteria. In these studies, the authors demonstrated increases in yields that were lower or comparable to those in the present study, *i.e.* about 12% after application of *Azospirillum brasilense* and about 25% after application of *Bacillus subtilis*. According to Reed and Glick (2023), the positive effects of bacteria on crops are due to a number of direct and indirect mechanisms. The increase in crop yield due to the application of *Azospirillum sp.* and *Azotobacter sp.* bacteria is most often attributed to BNF resulting in an increase in plant-available nitrogen in the soil (Ladha *et al*., 2022). These species also synthesize auxins, cytokinins, and gibberellins, which show positive effects on seed germination and root and shoot length in crop plants (Minuț *et al*., 2023). In turn, bacteria of the genus *Bacillus* promote plant growth through the secretion of metabolites and hydrolytic enzymes, such as cellulase, β-glucanase, and protease (Hashem *et al*., 2019). In addition, these bacteria can also solubilize phosphorus from the soil, increase nitrogen fixation, and produce siderophores that promote plant growth and inhibit pathogen activity (Hashem *et al*., 2019). According to Sivasakthi *et al*. (2014), bacteria of the genus *Pseudomonas* are an important group of microorganisms involved in disease control and promotion of crop plant growth. The positive effect of these bacteria on plants is attributed to the production of tailocin (Príncipe *et al*., 2018), lipopeptides, amphiphysin, pyrolnitrin, pyoluteorin, phenazine, tensin, or tropolone (Kundan *et al*., 2015) as well as indole-3-acetic acid (IAA) (Althaf *et al*., 2013) and ACC deaminase (Belimov *et al*., 2001). In the present research, the greatest increase in the spring barley yields and improvement of the yield structure was obtained after the simultaneous application of *Azotobacter chroococum*, *Pseudomonas fluorescens*, *Bacillus subtilis*, and *Bacillus amyloliquefaciens*. Other authors have also reported better crop yields after using co-inoculation (Naseri *et al*., 2013; de Aquino *et al*., 2023; Efthimiadou *et al*., 2020; Gaspareto *et al*., 2023). Research conducted by Gaspareto *et al*. (2023) using bacteria *Azotoacter salinestris* and *Azospirillum oryzea* in wheat and Shalaby *et al*. (2023) using bacteria *Azospirillum brasilense* and *Bacillus subtilis* in maize revealed the greatest increase in the number of grains per ear/cob and the weight of 1000 grains with the combined application of two bacterial formulations. In contrast, Voronina *et al*. (2024) demonstrated an increase in wheat ear length after bacterial inoculation and no significant differences in ear weight and 1000-grain weight, compared to the control group. In turn, Dawood *et al.* (2023) noted an increase in the number of grains per millet ear and the weight of 1000 grains as a result of the activity of *Enterobacter sp.* and *Stenothrofomonas maltophilia* bacteria. In their research, Khaliq *et al.* (2023) revealed improved wheat yield structure as a result of inoculation with *Bacillus sp.* and *Pseudomonas sp.* The authors also noted a significant increase in the length and weight of wheat roots after inoculation. The increased root weight is attributed to the improved grain yield structure as a result of inoculation with bacterial formulations. As a result of greater availability and more efficient uptake of nutrients by plants, there is an improvement in the yield structure of plants, which also directly translates into an increase in yield (de Andrade *et al.*, 2023). A possibility to provide plants with a wide range of plant growth-promoting mechanisms is the interaction of different microorganisms within a bacterial consortium (Zhang *et al.*, 2021; Santoyo *et al.*, 2021). According to Reed and Glick (2023), as individual PGPR have multiple mechanisms but not all of them are revealed in parallel in a specific environment. This is because the co-function of too many genes causes a metabolic strain on the bacteria, thus decreasing their overall environmental fitness (Glick, 1995). Thus, the use of bacterial consortia can provide multiple benefits specific to selected groups of bacteria simultaneously.

In our research, also the cultivation of spring barley with LM had a positive effect on the obtained grain yield and yield structure. However, on average, this effect was lesser in comparison with the use of the constructed proprietary bacterial consortia. Several studies demonstrated an increase in crop yield when LM was applied. Research by Liu *et al.* (2015) revealed an increase in barley yield by up to 4% when perennial ryegrass was grown with LM. In turn, Løes *et al.* (2011) demonstrated an average increase of 20% in grain yields when plants were grown with the LM of ryegrass, clover, and their mixtures. In contrast, in some field studies, the authors reported the opposite effects of cultivation with LM, *i.e.*, a reduction in the obtained grain yields (Bhaskar *et al.*, 2014; Känkänen and Eriksson, 2007). The contradictory reports on the effect of introducing LM to cereal crops may be due to several factors, primarily the type of crop used as LM and the timing of its sowing in relation to the main crop (Afshar *et al.*, 2018). The benefits of growing crops with LM are attributed to several mechanisms. As reported by Verret *et al.* (2017), LM can reduce the occurrence of weeds in crops by up to 52%, which is particularly important in organic farming. LM reduces weed infestation in main crops primarily due to soil cover thus creating competition for light for weeds (Petit *et al.*, 2018). The positive effect of LM on grain yield and yield structure can be attributed to improved soil quality (Gaudin *et al.*, 2013; Poeplau and Don, 2015). In addition, grass species, such as ryegrass, effectively draw nutrients from the soil preventing leaching (Poudel *et al.*, 2022). On the other hand, LM from legumes can positively influence crop grain yields and yield structure due to their ability to BNF (Abdul Rahman *et al.*, 2022). The mutual benefit seen in growing cereals with legume LM is due to differences in nitrogen acquisition (Nyfeler *et al.*, 2011). Legumes are generally less competitive than cereals or grasses in extracting nitrogen from the soil. Despite their ability to BNF with symbiosis with nodule bacteria, legumes, when nitrogen is available in the soil, will be more willing to use it because it is more energetically beneficial to the bacteria than BNF (Gastal and Lemaire, 2002). Thus, with cereal as the dominant crop, the cereal component will take up most of the available nitrogen in the soil, while the legume LM will rely on BNF (Cougnon *et al.*, 2022). In addition, bound nitrogen can be transferred from legumes to cereals by releasing organic and inorganic compounds from plant roots (Cougnon *et al.*, 2022). All in all, growing cereals with legume LM will help deliver more nitrogen to the cereals and thus increase the grain yield and yield structure. However, in growing cereals with LM, care should be taken to ensure minimal competition of the main crop with LM for limited resources (Radicetti *et al.*, 2018). This is achievable when species grown simultaneously occupy different niches in time and space (Malézieux *et al.*, 2009), which is achievable when legumes and grasses are used as LM.

In recent years, climate change has been observed, manifested in increased temperatures and water shortages, which negatively affect the yields of agricultural crops (Hafez *et al.*, 2021; Koryagin *et al.*, 2022). Also, in the present studies, the lower availability of precipitation during the spring barley growing season resulted in lower grain yields on average. Water deficiency causes a decrease in photosynthesis and negatively affects root metabolism (Sati *et al.*, 2023). Therefore, it is necessary to look for strategies to improve crop tolerance to water shortage (Slimani *et al.*, 2023a). Research conducted by Slimani *et al.* (2023b) demonstrated an increase in shoot and root dry weight as well as stomatal conductance and chlorophyll fluorescence of barley after the application of PGPR bacteria. According to Ortiz-Castro *et al.* (2020) and Bouremani *et al.* (2023), plants inoculated with PGPR bacteria show changes in the morphology and architecture of the root system resulting in increased nutrient and water transport under drought stress. The increased drought tolerance after PGPR bacteria application is attributed to the production of phytohormones, such as auxins (IAA), ACC deaminase (ACCd), cytokinins, abscisic acid (ABA), and gibberellins (GA) (Sati *et al.*, 2023). Auxins play a key role in plant development by controlling metabolic functions. Research by Karimi *et al.* (2020) proved improved water levels in wheat leaves inoculated with *Azospirillium sp.* as a result of IAA production. In turn, research by Raheem *et al.* (2018) demonstrated elongation and improvement of root trichome density as a result of IAA and ACCd synthesis by

Bacillus amyloliquefaciens. The authors also found a positive correlation between wheat yields and IAA production by *Bacillus thuringiensis*. Similarly, GA plays an important role in seed germination, stem elongation, flowering, and maturation. Thus, according to Khan *et al*. (2020), GA-synthesizing bacteria help plants become resistant to water stress. Cohen *et al*. (2009) demonstrated complete neutralization of drought effects in maize plants inoculated with *Azospirillium lipoferum* as a result of GA and ABA production. Cytokinins are hormones responsible for stem growth, plant cell division, photosynthesis, and regulation of stomatal opening during periods of drought. Zaheer (2019) demonstrated an increase in the level of cytokinins in wheat inoculated with *Azospirillum brasilense*, resulting in drought mitigation and higher grain yield. Also, several other studies (Rezaei *et al*., 2017; Rehman *et al*., 2022; Chandra *et al*., 2019) demonstrated that the application of PGPR bacteria increases grain yields in water shortage conditions. These reports are also confirmed by the present research.

In our research, the cultivation of spring barley with LM also had a positive effect on grain yield. The effect of LM was evident in both years with water shortage and years with higher precipitation. However, the average yield improvement effect of the cultivation with LM was slightly higher in a year with lower precipitation and comparable average temperature. Also, research by Sjursen *et al*. (2012) demonstrated an increase in grain yield in organic farming as a result of LM application in varying weather conditions. Sjursen *et al*. (2012) also demonstrated, similarly to the present research, the best effects when LM of clover and a mixture of clover and ryegrass were applied. The positive effect of introducing LM to crops, especially in adverse weather conditions, is attributed to several mechanisms. The LM fills the ecological niche by covering the soil, thereby reducing evaporation of water and limiting sunlight reaching the soil and thus reducing soil temperature (Teasdale *et al*., 2007). In addition, the cultivation of crops with LM makes it possible to achieve the maintenance of high levels of organic matter and biological activity in the soil; therefore, it provides the physical and chemical basis for resilient soils (Gaudin *et al*., 2013). Minimizing the impact of drought and high temperatures is possible as a result of capturing and storing water in soils that plants can use during periods of scarcity (Singer *et al*., 2006). Soils with high organic matter content show higher water holding capacity and infiltration rates (Bergström and Kirchmann, 2004). On the other hand, higher soil moisture content results in the maintenance of lower soil temperature for a longer period of drought, thus reducing water loss by plants (Gaudin *et al*., 2013). This is confirmed by research conducted by Boyd *et al*. (2001) on potatoes and Martin *et al*. (1999) on maize, in which soil moisture levels were at consistently higher levels as a result of cultivation with LM relative to cultivation without LM.

4.2. BIF

The soil microbiome is very important for the functionality of agroecosystems and biological processes occurring in the soil by regulating the transport of mineral and organic compounds (Fierer, 2017). Among other things, enzyme activity and physicochemical properties represent the dynamics of soil processes, thereby informing soil fertility (Gil-Sotres *et al*., 2005). Catalase and dehydrogenase are located in soil as essential elements of complete living microbial cells. Thus, they can be used as a measure of overall microbial activity in the soil and to determine the BIF (Sulewska *et al*., 2020). Higher BIF values indicate more fertile soil (Jaskulska *et al*., 2023). In our research, in all the three terms of soil BIF assessment, the use of the constructed proprietary consortia resulted in an increase in this indicator, but the highest values were obtained after the use of the consortium containing *Azotobacter chroococcum, Pseudomonas fluorescens, Bacillus subtilis*, and *Bacillus amyloliquefaciens* bacteria. Also, other authors demonstrated an increase in soil enzyme activity as a result of using bacterial formulations containing *Acinetobacter johnsonii, Bacillus altitudinis, Bacillus subtilis*, and *Bacillus altitudinis* (Shabaan *et al*., 2022), *Azospirillum lipoferum, Bacillus coagulans, Bacillus circulance,* and *Bacillus subtilis* (El-Shamy *et al*., 2022), or many other bacteria showing plant growth-promoting properties (Çakmakçı *et al*., 2023). The activity of rhizosphere microbes influences soil quality through their involvement in biochemical cycles and long-term soil balance (Newman *et al*., 2016). Thus, the increase in soil enzyme activity is due to chemical and physical changes in the soil, which should be linked to the microbial biomass of the soil (Balota *et al*., 2004). In our research, too, the cultivation of spring barley with LM caused an increase in BIF at all the analyzed dates. Also, research conducted by Elhawat *et al*. (2024) demonstrated a significant increase in dehydrogenase activity, while the difference observed in catalase activity was statistically insignificant with a decreasing trend. The increase in soil enzyme activity as a result of cultivation with LM can be attributed to root exudation, increased organic carbon content, and atmospheric nitrogen fixation by legumes, which interact with the soil as LM component plants (Qian *et al*., 2015). The activity of soil enzymes, according to Cirilli *et al*. (2012), depends on many factors including, but not limited to, soil moisture, temperature, and pH. Thus, the aforementioned ability of LM to maintain soil moisture and protect the soil from high temperatures certainly contributed to higher soil enzyme activity, which translated into a higher BIF value. This finding is corroborated by Elhawat *et al*. (2024) indicating that, among other things, increasing soil moisture creates better conditions for soil microbes and, consequently, improves their functions, such as soil enzyme activity. In our study, the value of BIF was increased between the tillering and flowering phases of spring barley, and the value obtained after harvesting spring barley was lower than in the flowering phase. Analogous relationships regarding BIF values were found in a study on spring wheat conducted by Niewiadomska *et al.* (2020). Also, a study by Mackiewicz-Walec *et al.* (2023) demonstrated higher dehydrogenase activity in the months coinciding with grain flowering, while the highest catalase activity was recorded in the months after grain harvest. On the other hand, in their study, Mandal *et al.* (2007) demonstrated a reduction in dehydrogenase activity at the flowering stage of wheat, compared to the tillering and soft dough stages. Thus, the data on soil enzyme activity available in the literature are inconclusive. This may be due to the fact that changes in BIF values and therefore dehydrogenase and catalase activities are influenced by several factors that are not necessarily related to the vegetative stage of plants. With the successive dates of the BIF analysis in our study, there was an increase in air temperature. As reported by Datt and Singh (2019), enzyme activity increases with an increase in temperature from low to medium and then decreases at higher temperatures above the optimum. Thus, it can be assumed that the increase in temperature between tillering and flowering of spring barley caused an increase in enzyme activity, and the high air temperature during the post-harvest soil sampling period was above the optimum, causing a decrease in enzyme activity. A similar relationship can be observed with precipitation. Droughts reduce soil enzyme production, resulting in low measured activity. In contrast, renewed water availability can lead to increased availability of organic matter, which in turn can result in a biomass turnover pulse for microorganisms, causing a temporary increase in enzyme activity (Datt and , 2019). This finding is confirmed by the present study, in which BIF increased with the increasing precipitation. In turn, Yousfi *et al.* (2021) found a highly significant correlation between soil organic matter content, plant root length, and catalase and dehydrogenase activity. Thus, it can be assumed that the increased organic matter content achieved by growing barley with LM has a positive effect on catalase and dehydrogenase activity. The gradual development of plants grown as LM between the tillering and flowering phases of spring barley could increase the organic matter content of the soil and the activity of soil enzymes. On the other hand, Siwik-Ziomek and Szczepanek (2019) suggest that soil enzyme activity is related to the rate of proliferation of soil microorganisms, which is usually the highest at the flowering stage. The authors also found a positive correlation between catalase activity and the amount of nitrogen uptake by plants, especially at maturity. In addition, Hupe *et al.* (2018) found that the stage of plant development has a significant influence on nutrient dynamics in the rhizosphere, and thus on soil enzymatic activity. The researchers observed the deposition of nitrogen and organic carbon in the rhizosphere during the period from plant emergence to flowering. Thus, it can be assumed that increased nutrient uptake during the flowering period of plants influences increased soil enzyme activity.

4.3. Correlation

According to Xu *et al.* (2018), the grain traits that determine grain yield are grain weight, number of grains per ear, and number of plants per unit area. This is confirmed by the results of our research, in which the highest correlation value was obtained between the spring barley grain yield and the number of grains per ear. In the present field experiment, the length of the ear, the weight of grains per ear, and the weight of one thousand grains were also correlated with the yield in descending order. Research conducted by other authors (Levakova, 2022; Singh *et al.*, 2015; Nikkhah *et al.*, 2010) also demonstrated a strong correlation between the analyzed yield parameters and barley grain yield. However, based on the research of the cited authors, it is not possible to clearly determine which yield parameter has the greatest influence on the obtained seed yield. The obtained highest value of the correlation coefficient between grain yield and yield traits was different each time. According to Levakov (2022), there is a relationship between the elements of spring barley grain yield structure: the formation of one of the elements can be compensated by the more significant development of the other in different conditions of the growing season. Also Xu *et al.* (2018) confirm that yield traits interact with each other. This could be a putative explanation for the different highest correlations between grain yield and given yield traits. The interaction of yield traits is also confirmed by the high correlation value obtained in our study.

The experiment in question also revealed a strong correlation between grain yield and BIF. Also Taheri *et al.* (2022) obtained a high correlation value between dehydrogenase and catalase and grain yield of the cereal plant. In our study, the greatest influence on barley grain yield was exerted by BIF at the grain flowering stage, which is due to the fact that cereals at the flowering and grain formation stage have the highest nutrient requirements. According to Zandi and Schnug (2022), the high value of dehydrogenase and catalase associated with soil microbial activity can improve the activity of many enzymes that support nutrient supply and plant growth.

5. CONCLUSIONS

The field experiment conducted suggests highly positive effects of using bacterial consortia and living mulch in the organic cultivation of spring barley. Cultivation in such a system shows positive effects due to the yield and yield structure of the crop regardless of weather conditions, which is extremely important due to the progressive climate change. Both the use of the bacterial consortia and living mulch additionally have a positive effect on the biological index of soil fertility value. The results of the research obtained allow us to conclude that the best cultivation results are achieved upon the application of a bacterial consortium containing *Azotobacter chroococcum, Pseudomonas fluorescens*, *Bacillus subtilis*, and *Bacillus amyloliquefaciens* together with the application of living mulch in the form of a mixture of red clover + Italian ryegrass. Thus, such a crop management technique can be recommended for use in widespread agricultural practice in organic farming.

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