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The Use of a Composition of Bacterial Consortia and Living Mulch to Reduce Weeds in Organic Spring Barley Cultivation as an Element of Sustainable Plant Production

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Abstract: Weed infestation of cereal crops in organic farming is becoming a serious problem in agriculture. Sustainable agriculture requires the search for and implementation of crop management techniques that will reduce weeds without negatively impacting the environment. This research refers to the principles of integrated plant protection in sustainable agriculture, allowing the use of chemical plant protection products to be limited to the absolute minimum. Technology for growing spring barley based on the use of bacterial consortia in combination with living mulch (LM) can be an interesting approach to this problem. The aim of this three-year field research was to determine the effects of bacterial consortia and LM on the level of weed infestation in the organic spring barley crop. Two factors were tested in the experiment: bacterial consortia factors: control (without bacterial consortia); 1—*Bacillus megaterium* var. *phosphaticum* and *Arthrobacter agilis*; 2—*Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Pseudomonas fluorescens*; and LM: control (without LM); red clover; red clover + Italian ryegrass; and Italian ryegrass. This research demonstrated that the bacterial consortia tested significantly reduced both the biomass and number of weeds, including the following dominant weeds: *Chenopodium album*, *Sinapis arvensis*, *Elymus repens*, and *Tripleurospermum inodorum*. The use of LM also significantly reduced the weed infestation of spring barley stands. The lowest biomass and number of weeds, with the exception of *Elymus repens*, were recorded on objects with LM Italian ryegrass in spring barley in combination with bacterial consortium 2. The introduction of cultivation with LM Italian ryegrass or its mixture with red clover and the use of bacteria should be recommended for the practice of sustainable agriculture, which will reduce weeds through an ecological method.

Keywords: weeds; plant growth-promoting rhizobacteria; living mulches; organic farming; sustainable agriculture

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1. Introduction

The occurrence of weeds is a major constraint on crops, so weed control is an essential element in any agricultural production system [1]. Every year, there are huge losses in the quantity and quality of crop yields caused primarily by the development of weeds, which are the most important biotic constraint on agricultural production. It is estimated that the uncontrolled growth and development of segregated species can cause up to 100% of crop losses in crops of economic importance. In intensive farming systems, weed control is based on the use of herbicides as the most effective method, but its prolonged and intensive use has unfriendly environmental impacts [2]. Their negative impact on ecosystems, as well as the introduction of legislation [3] regulating the use and reduction in the use of chemical pesticides, forced the development of a new way to control weeds in sustainable agriculture. Innovative and sustainable methods of controlling pests, including weeds, are therefore of paramount importance in modern approaches to integrated plant protection, which is a way of protecting plants from pest organisms involving the use of all available methods of plant protection, especially non-chemical methods (e.g., biocontrol) that minimize risks to human and animal health and the environment. The problem of reducing weed infestation on crops by non-chemical means is particularly important on organic farms and now also in sustainable crop production systems. However, it should be borne in mind that non-chemical methods of weed control are less effective [4]. Thus, agriculture based on a sustainable strategy and, especially, organic agriculture needs the use of appropriate crop varieties as well as management techniques to effectively overcome weed pressure, with limited use of crop protection products [5]. In addition, ongoing climate change is causing transformations in the weed species found in given areas [6]. Thus, weeds that were previously of low importance have become regionally important species [7–9]. As a result, crop ecosystems and agronomy are facing the need to adapt weed control methods to the changed conditions [6]. Thus, there is a need to look for crop management techniques, including organic crops, that provide opportunities to counteract crop weed infestation.

Biopreparations, derived from living organisms or their secondary metabolites, seem to be the ideal solution. High specificity and selectivity, low production costs, and harmlessness to the environment and humans make biological plant protection products a safe alternative to chemical pesticides. In recent years, bacterial formulations based on plant growth-promoting rhizobacteria (PGPR) have been increasingly widely and intensively used and researched in agriculture worldwide [10]. The widespread interest in this group of bacteria in agriculture is due, among other things, to their ability to positively influence the growth and development of crop plants [11]. PGPR support plant growth as a result of increasing the availability of soil nutrients, thereby increasing their absorption, synthesis of phytohormones, or secretion of enzymes [12]. Thus, the application of PGPR to crops generally leads to more intensive development and increased plant yield [13–15]. Indirectly, better plant development can increase the level of competition of crops against weeds, thereby reducing their occurrence. As reported by Mustafa et al. [16], some bacterial strains can act as bioherbicides by reducing weed germination and growth. Thus, inhibiting weed growth can also increase the competitive advantage of desirable plants [17]. Ongoing research in this area has also revealed that microorganisms can act very selectively, thereby eliminating specific weed species [18]. Identified rhizosphere bacteria showing bioherbicidal activities include species such as *Acinetobacter*, *Achromobacter*, *Alcaligenes*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Ralstonia*, *Serratia*, and *Rhizobium* [1,19,20]. However, the success rate of bacterial formulations can vary depending on the climate, soil conditions, or application site [21].

Another way of reducing weed infestation in crops, very well documented in the literature, is the use of living mulches (LMs) [22]. The mechanism of weed suppression by LM primarily involves the inhibition of weed germination and growth due to competition for resources such as light, water, and nutrients [23,24]. The introduction of LM into the main crop increases the degree of complementarity between crops, reducing the availability of resources to weeds, which is very importantly throughout the growing season [25]. The degree of weed suppression by LM is also often linked to rapid emergence and above- and below-ground development [26]. According to research by Vincent-Caboud et al. [27], growing season conditions and the type of plants applied can also influence weed suppression in proper crops. Thus, the selection of the appropriate plant species applied as LM seems to be crucial. The most commonly used plants as LMs are legumes and grasses [28]. However, when introducing LM into the main crop, care should be taken not to cause excessive competition with the main crop [29]. According to Bhaskar et al. [28], despite indisputable evidence of weed reduction as a result of LM application, their lack of widespread use is due to uncertainties about the best management practices.

Compiling the application of PGPR with LM can therefore provide the indicated positive effects of weed reduction in the main crop. In addition, their mutual influence can guarantee better results. Sjursen et al. [30] indicated a negative correlation between LM and weed biomass. Thus, better development of LM under the influence of PGPR may increase weed suppression.

The aim of this three-year field research was to appraise the impact of applying bacterial consortia and LM in organic spring barley cultivation on the weight, number, and composition of dominant weeds. The research hypothesis was that both the bacterial consortia and LM would reduce weed infestation in the spring barley crop, and their appropriate combination would identify the most effective management technique for the organic crop.

2. Materials and Methods

2.1. Field Research Location

Field research was carried out in Poland in a temperate climate during 2019–2021 on an organic farm located near Siedlce (52°12′35″ N, 22°11′05″ E). Soil conditions during field research are presented in Table 1, and weather conditions presented in Figure 1.

Table 1. Soil conditions when conducting field research.

Factor	**Years**			**Means**
	2019	**2020**	**2021**	
pH	6.1	6.1	6.2	6.1
Organic carbon (% d.m.)	1.04	1.04	1.07	1.05
P (mg 100 g^{-1} soil)	8.2	8.4	8.3	8.3
K (mg 100 g^{-1} soil)	12.1	12.2	12.0	12.1
Mg (mg 100 g^{-1} soil)	4.1	4.1	4.4	4.2

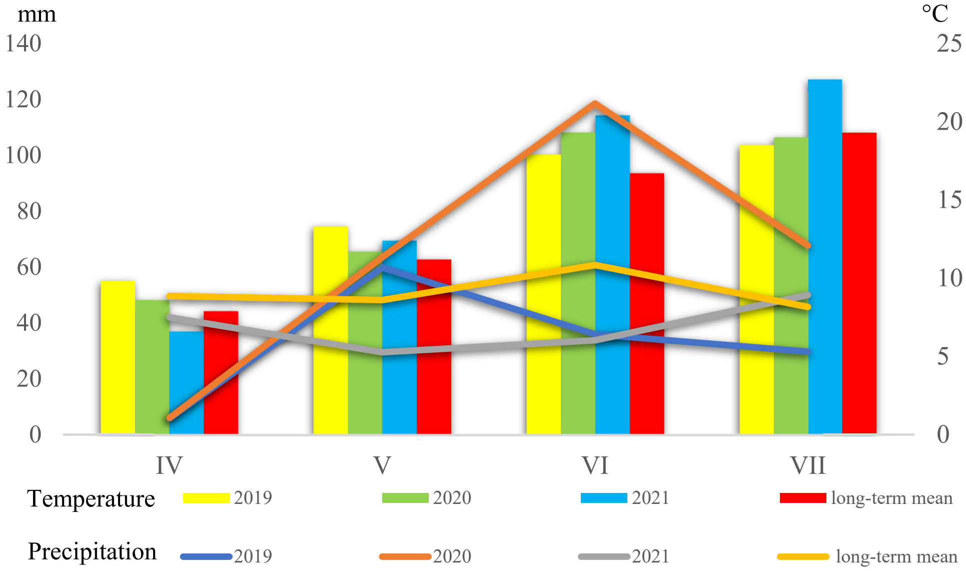

Figure 1. Weather conditions during the conduct of the field research.

2.2. Cultivation Management and Data Collection

The field experiment was conducted in three replicates. The forecrop for spring barley was winter rye. The area of one experimental object was 20 m^2. Two factors of experience were analyzed: the use of bacterial consortia, including *Bacillus megaterium* var. *phosphaticum*, *Arthrobacter agilis*, *Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Pseudomonas* *fluorescens*, and cultivation with LM, including red clover, Italian ryegrass and mixtures, and red clover + Italian ryegrass. A scheme of one repetition of the field experiment and management technique is shown in Figure 2.

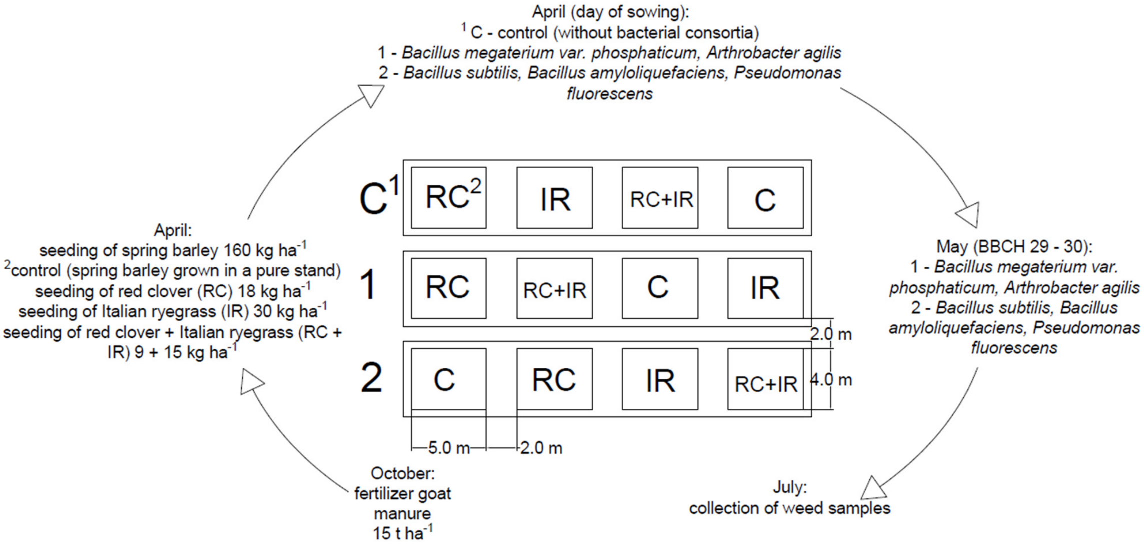

Figure 2. A schematic diagram of the field experiment. ^{1} C—control (without bacterial consortia); 1—*Bacillus megaterium* var. *phosphaticum*, *Arthrobacter agilis*; 2—*Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Pseudomonas fluorescens*; ^{2} C—control (spring barley grown in a pure stand); RC—red clover; IR−Italian ryegrass.

On objects where spring barley was grown with LM, no weed control treatments were applied. On objects where spring barley was cultivated without LM, two mechanical treatments were executed. The initial treatment, employing a light harrow, was carried out post-emergence. The second treatment, utilizing a medium harrow, was administered after the plants had developed 5–6 leaves. The bacterial species used for inoculation were sourced from the collection of the Department of Soil Science and Microbiology at the University of Life Sciences in Poznań. The isolates were obtained from cultivated plants using a selective medium, and their genetic identification was performed based on a fragment of their 16S rRNA gene sequence. The bacterial consortium had a cell density of 10^8 CFU mL^{-1} and was applied at a rate of 1 L per 250 L of water per hectare using a hand sprayer. Bacterial consortia were used on experimental objects on a cloudy, warm day with temperatures between 18 and 25 °C. The harvesting of spring barley was carried out at the end of July. Just before harvest, weed samples were taken from two randomly selected locations of each object delineated by a 1.0 × 0.5 m frame to determine fresh and dry weight, as well as number and species composition.

2.3. Statistical Analysis

Statistical analysis was performed with a three-way analysis of variance (ANOVA) using Statistica software version 13.3 (Hamburg, Germany). The significance of sources of variation was determined using Fisher–Snedecor's F test ($p \leq 0.05$). Tukey's HSD post-hoc test was used to determine differences between means ($p \leq 0.05$). Pearson's correlation coefficient was calculated to assess the strength of the linear relationship between DM, FM, weed number, and spring barley grain yield.

3. Results

3.1. Fresh Matter of Weeds

The bacterial consortia used in the field research had a significant ($p < 0.001$) influence on the fresh matter (FM) of weeds at the harvest of spring barley (Table 2).

Table 2. The fresh matter of weeds in spring barley as affected by bacterial consortia and living mulch (means across 2019–2021), g m^{-2}.

Bacterial Consortia	LM				Means
	Control	Red Clover	Red Clover + Italian Ryegrass	Italian Ryegrass	
Control	284.9 ± 22.4 a [2]	136.9 ± 17.0 b	103.0 ± 19.4 c	76.0 ± 16.6 c	150.2 ± 82.9 A
1 [1]	156.2 ± 16.7 a	82.7 ± 14.2 b	69.3 ± 12.9 b	52.3 ± 15.5 b	90.1 ± 42.7 B
2	111.3 ± 16.2 a	62.1 ± 17.2 b	52.4 ± 15.3 b	46.9 ± 16.7 b	68.2 ± 30.6 C
Means	184.1 ± 75.9 A	93.9 ± 35.4 B	74.9 ± 27.7 C	58.4 ± 20.6 D	
p-values	*bacterial consortia: <0.001; LM: <0.001 bacterial consortia × LM: <0.001*				

[1] 1—*Bacillus megaterium* var. *phosphaticum* and *Arthrobacter agilis*, 2—*Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Pseudomonas fluorescens*; [2] different small letters (a, b, and c) indicate significant differences ($p \leq 0.05$) for interactions; different capital letters (A, B, C, and D) indicate significant differences ($p \leq 0.05$) for main effects; ±standard deviation.

The highest average FM of weeds was determined on control objects where bacterial consortia were not applied. The application of bacterial consortium 1 reduced the FM of weeds by 40%. On the other hand, the lowest average FM of weeds was observed with bacterial consortium 2. In this variant, a reduction of 55% was observed in relation to the control objects. Also, growing spring barley with LM significantly ($p < 0.001$) differentiated the FM of weeds (Table 2). The highest FM of weeds was revealed on the objects where LM was not applied. According to the comparison with control objects, the application of LM of red clover reduced the FM of weeds by 49%, LM of a mixture red clover + Italian ryegrass by 59%, while LM of Italian ryegrass by 68%. A significant ($p < 0.001$) interaction of bacterial consortia x LM was also demonstrated (Table 2). In the absence of the use of bacterial consortia, the highest FM of weeds was characterized by control objects without LM, significantly lower objects where the LM was red clover, and significantly lowest objects where the LM of a mixture red clover + Italian ryegrass and LM of Italian ryegrass was applied. When bacterial consortia 1 and 2 were applied, the highest FM of weeds was also recorded on objects without LM. On the other hand, the objects on which LM was applied significantly showed the lowest FM of weeds.

In the field research conducted, the growing season conditions also had a significant ($p < 0.001$) influence on the FM of weeds at the harvest of spring barley (Figure 3).

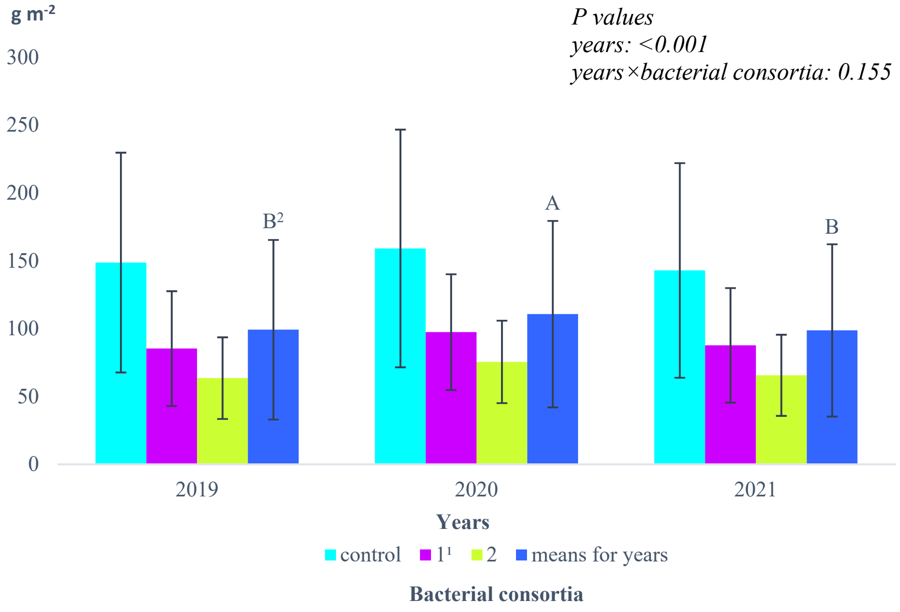

Figure 3. The fresh matter of weeds in spring barley as affected by bacterial consortia in 2019–2021, g·m^{-2}. [1] 1—*Bacillus megaterium* var. *phosphaticum* and *Arthrobacter agilis*, 2—*Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Pseudomonas fluorescens*; [2] different capital letters (A, B) indicate significant differences ($p \leq 0.05$) for years; ±standard deviation.

The highest FM of weeds was recorded in 2020, while it was significantly lower in 2019 and 2021. With respect to 2020, 2019 had a lower FM of weeds by 10% and 2021 by 11%. On the other hand, statistical analysis demonstrated the absence of a significant (p = 0.155) interaction of years x bacterial consortia. However, an apparent trend of lower FM of weeds in all years of research after the influence of bacterial consortia application can be observed.

There was also no significant interaction (p = 0.051) of growing season conditions × LM (Figure 4).

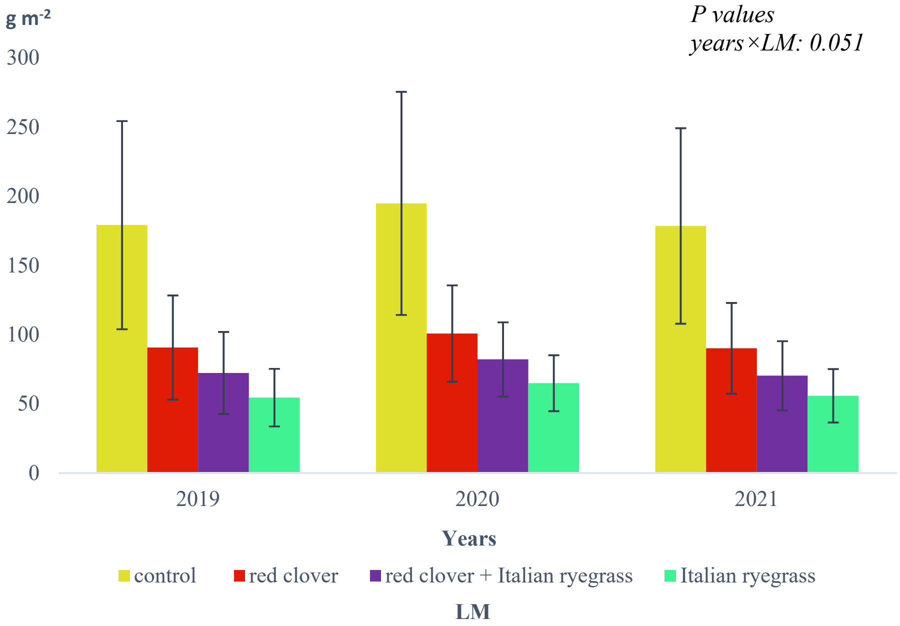

Figure 4. The fresh matter of weeds in spring barley as affected by living mulch in 2019–2021, g·m^{-2}; ±standard deviation.

However, there was also a trend in all years of research. The highest FM of weeds was revealed on the objects when spring barley was grown without LM. On the other hand, all applied LM resulted in a lower FM of weeds.

3.2. Dry Matter of Weeds

Inoculation with engineered bacterial consortia of a spring barley crop produced significant differences ($p < 0.001$) in the dry matter (DM) of weeds (Table 3).

Table 3. The dry matter of weeds in spring barley as affected by bacterial consortia and living mulch (means across 2019–2021), g m^{-2}.

Bacterial Consortia	LM				Means
	Control	Red Clover	Red Clover + Italian Ryegrass	Italian Ryegrass	
Control	95.1 ± 18.5 a [2]	45.8 ± 10.1 b	34.3 ± 7.9 c	25.3 ± 2.3 c	50.1 ± 27.1 A
1 [1]	52.1 ± 13.2 a	25.6 ± 8.4 b	23.2 ± 8.5 b	17.4 ± 1.9 b	29.6 ± 13.4 B
2	37.2 ± 7.6 a	20.8 ± 5.1 b	17.4 ± 2.3 b	15.8 ± 1.8 b	22.8 ± 8.7 C
Means	61.4 ± 24.8 A	30.7 ± 11.0 B	25.0 ± 9.1 C	19.5 ± 4.5 D	
p-values	*bacterial consortia:* <0.001; *LM:* <0.001 *bacterial consortia* × *LM:* <0.001				

[1] 1—*Bacillus megaterium* var. *phosphaticum* and *Arthrobacter agilis*, 2—*Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Pseudomonas fluorescens*; [2] different small letters (a, b, and c) indicate significant differences ($p \leq 0.05$) for interactions; different capital letters (A, B, C, and D) indicate significant differences ($p \leq 0.05$) for main effects; ±standard deviation.

The highest average DM of weeds was characterized by objects where no bacterial consortia were applied. The application of bacterial consortium 1 reduced weed DM by

41% and bacterial consortium 2 by 54%. Also, growing spring barley with LM resulted in significant differences ($p < 0.001$) in the DM of weeds at the harvest date of the main crop (Table 3). The significantly highest DM of weeds was characterized by objects where spring barley was grown without LM. The application of LM resulted in significantly lower weed DM. With respect to control objects, the LM of red clover induced a reduction in weed DM by 50%, the LM of a mixture of red clover + Italian ryegrass by 59%, and the LM of Italian ryegrass by 68%. A significant ($p < 0.001$) interaction of bacterial consortia x LM was also revealed (Table 3). In the absence of the use of bacterial consortia, the highest DM of weeds was characterized by objects where spring barley was cultivated without LM. In contrast, the introduction of red clover into the spring barley LM crop resulted in a significant reduction in weed DM. The lowest DM of weeds was demonstrated on objects where spring barley was cultivated with the LM of a mixture red clover + Italian ryegrass and the LM of Italian ryegrass. The use of bacterial consortia 1 and 2 in the spring barley cultivation also revealed the highest DM of weeds in the combination grown without LM. In contrast, the significantly lowest DM of weeds did not differ significantly on the other objects with all types of LM.

Growing season conditions also had a significant ($p < 0.001$) influence on weed DM (Figure 5).

The highest DM of weeds was recorded in 2020, while it was significantly lower in 2019 and 2021. With respect to 2020, in 2019, the DM of weeds was 10% lower, while in 2021, it was 11% lower. For the DM of weeds, no significant ($p = 0.113$) interaction of years × bacterial consortia was revealed (Figure 5). On the other hand, similar to weed FM, weed DM also showed a tendency to decrease under the application of bacterial consortia. On the other hand, a significant interaction ($p < 0.001$) of growing season conditions x LM was revealed (Figure 6).

It shows that in all years of the field experiment, the highest DM of weeds was recorded on objects where spring barley was grown without LM. In contrast, the application of LM caused a significant reduction in weed DM, analogous in all years of research. In ascending order of weed DM reductions, LM was as follows: red clover, red clover + Italian ryegrass mixture, and Italian ryegrass.

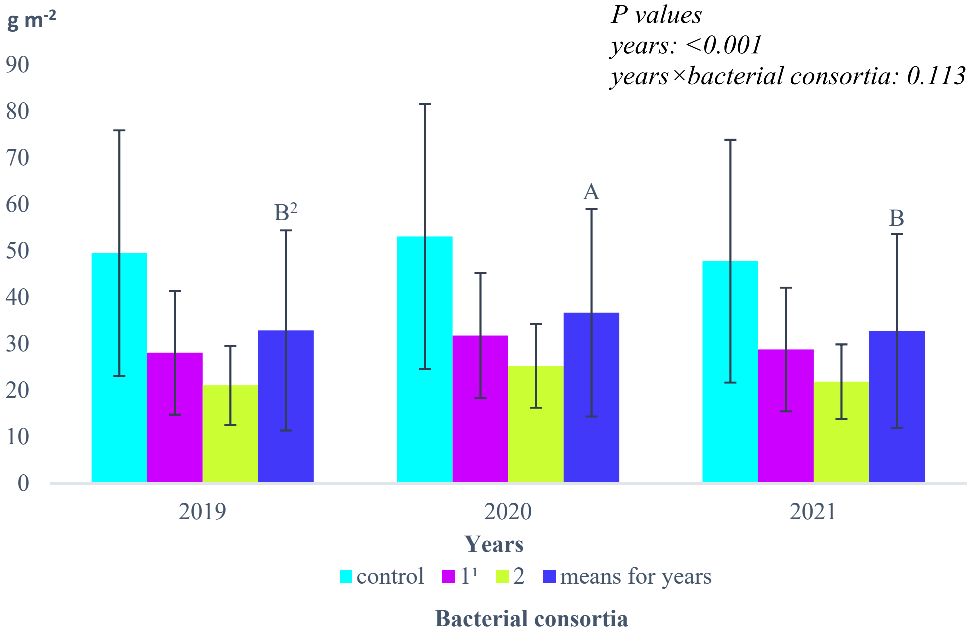

Figure 5. The dry matter of weeds in spring barley as affected by bacterial consortia in 2019–2021, g m^{-2}. [1] 1—*Bacillus megaterium* var. *phosphaticum* and *Arthrobacter agilis*, 2—*Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Pseudomonas fluorescens*; [2] different capital letters (A and B) indicate significant differences ($p \leq 0.05$) for years; ±standard deviation.

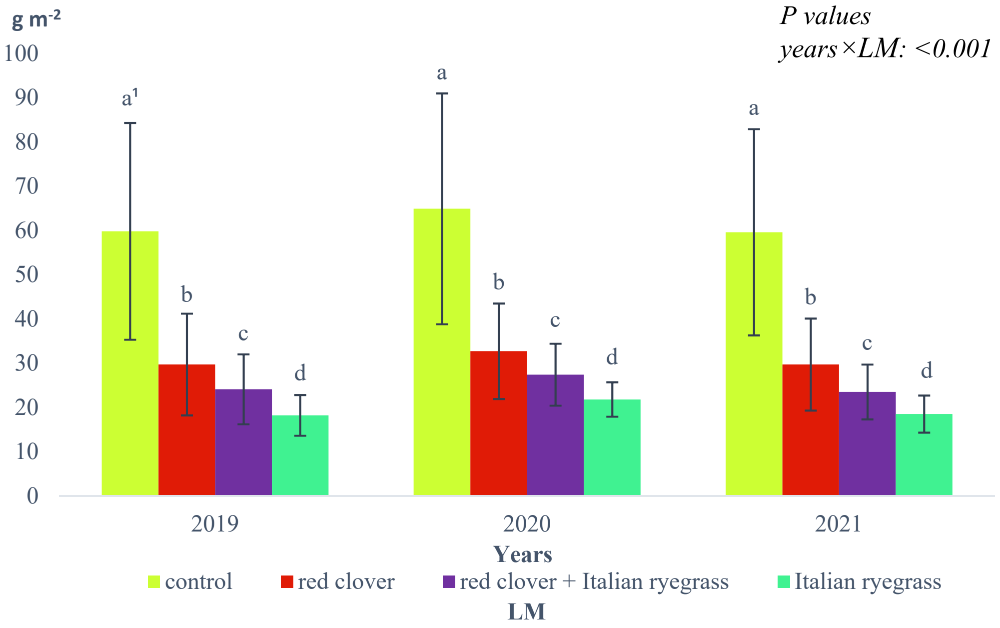

Figure 6. The dry matter of weeds in spring barley as affected by living mulch in 2019–2021, g m^{-2}. [1] Different small letters (a, b, c, and d) indicate significant differences ($p \leq 0.05$) for interactions; ±standard deviation.

3.3. Number of Weeds

The number of weeds at harvest time in spring barley was significantly ($p < 0.001$) differentiated by the use of bacterial consortia (Table 4).

Table 4. The number of weeds in spring barley as affected by bacterial consortia and living mulch (means across 2019–2021), pcs. m^{-2}.

Bacterial Consortia	**LM**				**Means**
	Control	**Red Clover**	**Red Clover + Italian Ryegrass**	**Italian Ryegrass**	
Control	66.4 ± 33.8 a [2]	32.5 ± 20.1 b	29.0 ± 15.4 b	23.6 ± 14.8 b	37.9 ± 19.8 A
1 [1]	27.7 ± 15.7 a	16.9 ± 10.0 ab	13.8 ± 8.8 b	14.1 ± 6.9 b	18.1 ± 9.1 B
2	23.2 ± 13.1 a	11.6 ± 7.8 b	13.2 ± 9.1a b	11.6 ± 7.1 b	14.9 ± 9.4 C
Means	39.1 ± 20.8 A	20.3 ± 11.9 B	18.7 ± 10.6 C	16.4 ± 11.2 D	
p-values	*bacterial consortia*: <0.001; *LM*: <0.001 *bacterial consortia* × *LM*: <0.001				

[1] 1—*Bacillus megaterium* var. *phosphaticum* and *Arthrobacter agilis*, 2—*Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Pseudomonas fluorescens*; [2] different small letters (a and b) indicate significant differences ($p \leq 0.05$) for interactions; different capital letters (A, B, C, and D) indicate significant differences ($p \leq 0.05$) for main effects; ±standard deviation.

The highest number of weeds was recorded on objects where bacterial consortia were not applied. The use of bacterial consortium 1 caused a significant reduction in the number of weeds by 52% and bacterial consortium 2 by 61% regarding the lack of use of bacterial consortia. The number of weeds was also significantly ($p < 0.001$) influenced by the LMs applied to the spring barley (Table 4). The lack of application of LM resulted in the highest number of weeds at the harvest of spring barley. The introduction of red clover into the main crop of LM resulted in a significant reduction in weed numbers by 48%. Subsequently, the LM of a mixture of red clover and Italian ryegrass resulted in a 52% lower weed number. The lowest number of weeds was revealed on objects where the LM was Italian ryegrass. With respect to objects without LM, the number of weeds was lower by 58%. A significant ($p < 0.001$) interaction of bacterial consortia x LM was also demonstrated (Table 4). On objects where bacterial consortia were not applied, the highest number of weeds was revealed in the absence of LM. In contrast, the application of LM, regardless of its type, allowed a significant decrease in the number of weeds. In the case of objects where bacterial consortium 1 was applied, the highest number of weeds was also found on objects without LM, while additionally, no statistically significant difference was found between control objects and objects with the LM of red clover. The lowest number of weeds was characterized by objects where the LM was a mixture of red clover + Italian ryegrass and Italian ryegrass, but in this case, there was no significant difference between objects where the LM of red clover was used. On objects where bacterial consortium 2 was used, the highest number of weeds was also demonstrated on objects without LM; in addition, no significant difference was found between this object and the use of the LM of a mixture red clover + Italian ryegrass. The smallest number of weeds was found after the application of the LM red clover and LM Italian ryegrass in the main crop, but in these cases, no significant difference was found in relation to the LM mixture.

Growing season conditions also significantly ($p < 0.001$) influenced the number of weeds in the spring barley crop (Figure 6). The highest number of weeds was recorded in 2020, with a significantly lower number in 2021 and the lowest in 2019. With respect to 2020, the number of weeds was 62% lower in 2021 and 75% lower in 2019. A significant ($p < 0.001$) interaction of field experiment success years × bacterial consortia was also demonstrated (Figure 7).

In all analyzed growing seasons, the highest number of weeds was found on objects without the use of bacterial consortia, less after the use of consortium 1, and at the lowest level on objects where bacterial consortium 2 was used. A significant ($p < 0.001$) interaction of growing season conditions × LM (Figure 8) was also revealed. It demonstrates that in all years of conducting the field experiment, the highest number of weeds was characterized by objects on which LM was not introduced into the main crop. The application of LM reduced the number of weeds with increasing effectiveness: the LM of red clover, the LM of a mixture of red clover + Italian ryegrass, and the LM of Italian ryegrass.

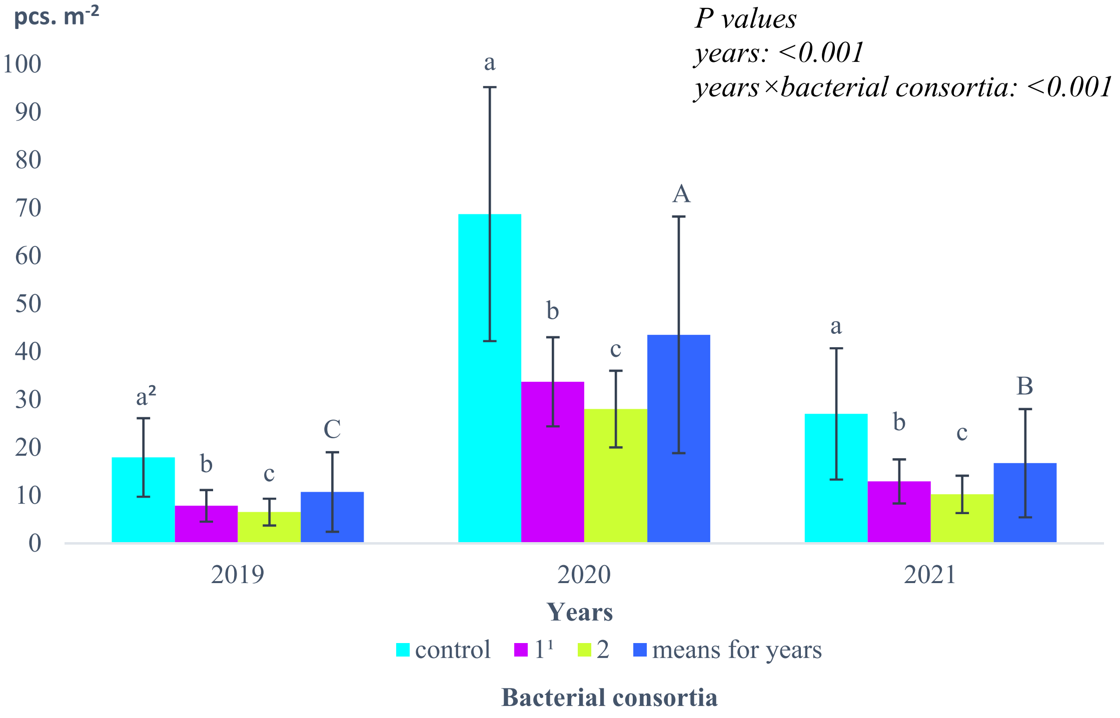

Figure 7. The total number of weeds in spring barley as affected by bacterial consortia in 2019–2021, pcs. m^{-2}. [1] 1—*Bacillus megaterium* var. *phosphaticum* and *Arthrobacter agilis*, 2—*Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Pseudomonas fluorescens*; [2] different small letters (a, b, and c) indicate significant differences ($p \leq 0.05$) for interactions; different capital letters (A, B, and C) indicate significant differences ($p \leq 0.05$) for years; ±standard deviation.

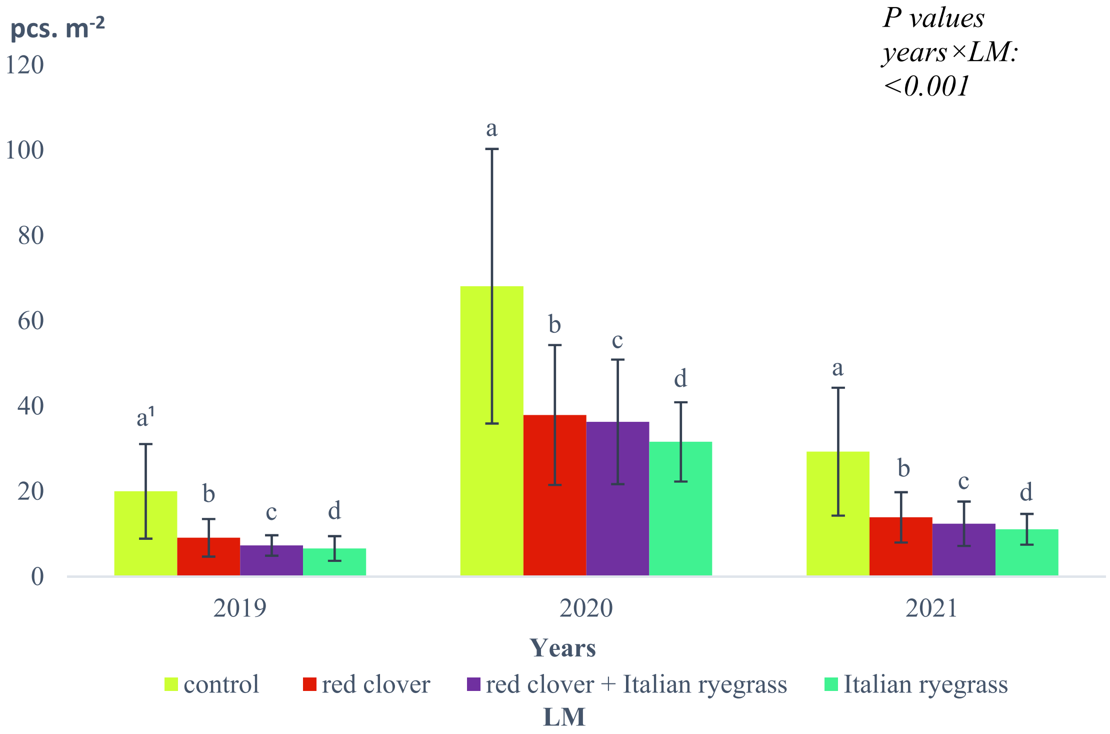

Figure 8. The number of weeds in spring barley as affected by living mulch in 2019−2021, pcs. m^{-2}. [1] Different small letters (a, b, c, and d) indicate significant differences ($p \leq 0.05$) for interactions; ±standard deviation.

A correlation analysis demonstrated a significant negative effect of the FM (p = 0.002) and DM (p = 0.006) of weeds on the obtained yield of spring barley. In contrast, the correlation of the number of weeds with the yield of spring barley was at a non-significant statistical level (Table 5).

Table 5. Correlation coefficients between the analyzed characteristics.

	Spring Barley Yield	*p*-Values
FM of weed	−0.2615	*0.002*
DM of weed	−0.2919	*0.006*
Number of weeds	0.0933	*0.337*

3.4. Dominant Weed Species

The dominant weed species in the organic spring barley were *Chenopodium album*, *Sinapis arvensis*, *Tripleurospermum inodorum*, and *Elymus repens* (Table 6).

For all dominant weed species, there was a significant (p < 0.001) effect of the application of bacterial consortia on their number (Table 6). The highest number of dominant weeds was found in the absence of the use of bacterial consortia. As for the quantity of *Chenopodium album*, the use of bacterial consortia caused a significant, not different, reduction of 63% after the use of bacterial consortium 1 and 66% after the use of bacterial consortium 2. As for the quantities of *Sinapis arvensis* and *Tripleurospermum inodorum*, significantly greater reductions were observed on objects where bacterial consortium 2 was applied than 1. For *Sinapis arvensis*, there was a decrease in numbers of 41% for consortium 1 and 61% for consortium 2 relative to control objects. For *Tripleurospermum inodorum*, on the other hand, the use of bacterial consortium 1 resulted in a 48% reduction in numbers and 58% for bacterial consortium 2 relative to control objects. A higher efficiency of reducing the quantity of *Elymus repens* in spring barley differently from the other dominant species was observed after the use of bacterial consortium 1 than 2. The reduction in the quantity of *Elymus repens* after the use of bacterial consortium 1 was 57% and bacterial consortium 2 was 48%. Also, the introduction of LM into the spring barley crop had a significant (p < 0.001) influence on the numbers of all dominant weeds (Table 6). The highest numbers of *Chenopodium album*, *Sinapis arvensis*, and *Tripleurospermum inodorum* were recorded on control objects without LM. The LMs applied to the main crop induced a significant decrease in the number of the indicated dominant weeds with successively increasing effectiveness: the LM of red clover, the LM of a mixture red clover + Italian ryegrass, and the LM of Italian ryegrass. The LM of red clover reduced the occurrence of *Chenopodium album* by 53%, the LM of a mixture red clover + Italian ryegrass by 64%, and the LM of Italian ryegrass by 76%. In contrast, the incidence of *Sinapis arvensis* in the spring barley crop was reduced by the LM of red clover by 54%, the LM of a mixture red clover + Italian ryegrass by 62%, and the LM of Italian ryegrass by 72%. On the other hand, the quantity of *Tripleurospermum inodorum* decreased by 54% with the LM of red clover, 59% with the LM of a mixture red clover + Italian ryegrass, and by 78% for objects with the LM of Italian ryegrass. A different trend was observed for *Elymus repens*. With respect to the control objects, the LM of red clover caused a reduction in incidence of only 6%. Statistically, these objects did not differ. In contrast, the introduction of the LM of red clover + Italian ryegrass mixtures into the spring barley crop resulted in a 27% increase in *Elymus repens*, while the LM of Italian ryegrass increased by 46%. It was on objects where Italian ryegrass LM was applied that the highest average quantity of *Elymus repens* was revealed significantly. A significant (p < 0.001) interaction of bacterial consortia × LM was also demonstrated (Table 6). The highest quantity of *Chenopodium album* in the absence of the use of bacterial consortia was found with the combination with no LM. Significantly lower numbers were found with the LM of red clover and the LM of a mixture of red clover + Italian ryegrass. The significantly lowest number of *Chenopodium album* was determined with the LM of Italian ryegrass; in addition, there was no statistical difference between these objects and the objects where the LM was a mixture red clover + Italian ryegrass. Bacterial consortium combination 1 with both no LM and all tested LM demonstrated no statistical difference in the number of *Chenopodium album*. In contrast, on objects where bacterial consortium 2 was used, the highest number of *Chenopodium album* was also found in the absence of LM, but it was not significantly different from objects where the LM was red clover. A lower incidence of this weed species was found on objects where the LM of a mixture red clover + Italian ryegrass and the LM of Italian ryegrass was applied, but in these cases, no significant differences were found with objects where the LM was red clover. For the occurrence of *Sinapis arvensis* in the crop in the absence of the application of bacterial consortia, the highest number was revealed in the absence of LM, significantly lower for the LM of red clover and the LM of a mixture red clover + Italian ryegrass, and significantly lowest when the LM was Italian ryegrass. On objects where bacterial consortiums 1 and 2 were used, the highest number of *Sinapis arvensis* was also recorded on objects without LM, while it was significantly lower on all objects where LM was introduced regardless of its type. The highest number of *Tripleurospermum inodorum* on objects where bacterial consortia were not applied was determined in the absence of LM. The use of a combination of no bacterial consortia with LM significantly reduced the incidence of *Tripleurospermum inodorum*, regardless of the type of LM. On objects using bacterial consortium 1, non-statistically different numbers of *Tripleurospermum inodorum* were found on objects without LM, with the LM of red clover, and with the LM of a mixture of red clover and Italian ryegrass. In contrast, a significantly lower population of *Tripleurospermum inodorum* was characterized by objects on which Italian ryegrass was the LM. On the other hand, when bacterial consortium 2 was applied to all objects with LM and no LM, the number of *Tripleurospermum inodorum* was not statistically different. In the case of the occurrence of *Elymus repens* in the spring barley crop in the absence of bacterial consortia, the highest number of this weed species was found on objects with Italian ryegrass LM, lower with the LM of red clover + Italian ryegrass mixture, and significantly the lowest with the LM of red clover and in the absence of LM. On the other hand, after the use of bacterial consortium 1, statistically the same level of

Elymus repens was found in all tested combinations of LM and no LM. However, after the use of bacterial consortium 2, the highest number of *Elymus repens* was also found when Italian ryegrass was introduced into the main crop of LM. However, it was not significantly different from that obtained when the LM was a mixture of red clover + Italian ryegrass. A significantly lower number of *Elymus repens* was determined on the objects with the LM of red clover, while in this case no statistical differences were revealed by the objects of the LM of the mixture. In contrast, the lowest number of this weed species was determined when bacterial consortium 2 was applied to spring barley crops without LM. In addition, however, the number of *Elymus repens* on these objects was not significantly different from objects when the LM was red clover.

Table 6. The number of dominant weed species in spring barley as affected by bacterial consortia and living mulch (means across 2019–2021), pcs. m^{-2}.

Bacterial Consortia	**LM**	*Chenopodium album*		*Sinapis arvensis*		*Tripleurospermum inodorum*		*Elymus repens*	
			Means for Bacterial Consortia		**Means for Bacterial Consortia**		**Means for Bacterial Consortia**		**Means for Bacterial Consortia**
Control	Control	24.4 ± 14.0 a [2]	11.9 ± 8.7 A	19.4 ± 8.1 a	10.2 ± 8.5 A	13.4 ± 8.0 a	6.2 ± 3.5 A	4.9 ± 3.4 c	6.3 ± 4.8 A
	Red clover	11.0 ± 5.9 b		9.6 ± 7.5 b		4.8 ± 3.2 b		4.1 ± 3.2 c	
	Red clover + Italian ryegrass	8.2 ± 5.9 bc		7.4 ± 5.5 b		4.0 ± 3.1 b		6.9 ± 4.9 b	
	Italian ryegrass	4.1 ± 2.5 c		4.6 ± 3.1 c		2.6 ± 1.8 b		9.5 ± 5.5 a	
1 [1]	Control	6.3 ± 4.4 a	4.4 ± 2.9 B	11.3 ± 5.3 a	6.0 ± 4.9 B	5.3 ± 3.8 a	3.2 ± 2.4 B	2.4 ± 1.7 a	2.7 ± 2.1 C
	Red clover	4.4 ± 2.4 a		5.4 ± 3.7 b		3.4 ± 2.2 a		2.3 ± 1.7 a	
	Red clover + Italian ryegrass	3.6 ± 2.2 a		3.6 ± 2.8 b		2.6 ± 1.7 a		2.6 ± 1.5 a	
	Italian ryegrass	3.4 ± 2.4 a		3.7 ± 2.4 b		1.4 ± 0.6 b		3.5 ± 2.6 a	
2	Control	8.6 ± 5.3 a	4.1 ± 3.0 B	7.4 ± 5.1 a	4.0 ± 2.8 C	4.2 ± 2.8 a	2.6 ± 1.9 C	2.3 ± 1.7 c	3.3 ± 2.8 B
	Red clover	3.2 ± 2.2 ab		2.5 ± 1.7 b		2.3 ± 1.7 a		2.6 ± 1.9 bc	
	Red clover + Italian ryegrass	2.4 ± 1.7 b		3.4 ± 2.2 b		2.6 ±1.9 a		3.7 ± 2.6 ab	
	Italian ryegrass	2.0 ± 0.9 b		2.5 ± 1.7 b		1.2 ± 0.9 a		4.7 ± 3.7 a	
Means for LM	Control	13.1 ± 8.2 A		12.7 ± 8.0 A		7.6 ± 4.8 A		3.2 ± 2.7 C	
	Red clover	6.2 ± 4.2 B		5.8 ± 4.1 B		3.5 ± 2.7 B		3.0 ± 2.4 C	
	Red clover + Italian ryegrass	4.7 ± 2.1 C		4.8 ± 3.6 C		3.1 ± 2.5 C		4.4 ± 3.6 B	
	Italian ryegrass	3.2 ± 2.2 D		3.6 ± 2.7 D		1.7 ± 1.2 D		5.9 ± 4.8 A	
p-values	*Bacterial consortia*	<0.001		<0.001		<0.001		<0.001	
	LM	<0.001		<0.001		<0.001		<0.001	
	Bacterial consortia × LM	<0.001		<0.001		<0.001		<0.001	

[1] 1—*Bacillus megaterium* var. *phosphaticum* and *Arthrobacter agilis*, 2—*Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Pseudomonas fluorescens*; [2] different small letters (a, b, and c) indicate significant differences ($p \leq 0.05$) for interactions; different capital letters (A, B, C, and D) indicate significant differences ($p \leq 0.05$) for main effects; ±standard deviation.

4. Discussion

An important problem found in organic and sustainable agriculture is the control of weeds in the main crops, which, in the case of the former, requires a total non-usage and, in the case of the latter, a partial reduction in the use of chemical pesticides [31]. The problem is primarily due to the much lower effectiveness of non-chemical methods of weed control compared to chemical methods [4]. Therefore, organic crops generally show a higher level of weed infestation compared to conventional crops [32]. This is consistent with the results of our own research, in which a high level of weed infestation was revealed on control objects. Research by Wacłowicz et al. [33] and Mahajan et al. [34] demonstrated a negative correlation between morphological traits of spring barley and the biomass and number of weeds. Excessive weed growth can cause a reduction in the development of the main crop, thereby causing yield losses [24,25]. This was confirmed by Mason et al. [35], who demonstrated a negative correlation between weed biomass and wheat yield. These findings are consistent with those obtained in our own research, in which a significant negative correlation was demonstrated between the FM and DM of weeds and spring barley yield.

Thus, it is right to look for management techniques that can reduce weed infestation in organic crops and can also be a component for use in sustainable agriculture. According to Mustafa et al. [16], one potential method of reducing weed incidence in main crops may be the use of microbial preparations. In our own research, the use of bacterial consortia containing *Bacillus meganterium* var. *phosphaticum*, *Artrobacter agilis*, *Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Pseudomonas fluorescences* reduced the incidence of weed mass and number in the organic spring barley crop. Also, in studies by other authors, a reduction in weed infestation was found. Research by Abbas et al. [36] demonstrated a reduction in weed infestation in rice as a result of *Pseudomonas fluorescences*. Analogous results after the application of four strains of *Pseudomonas* spp. in wheat crops were also obtained by Dar et al. [37]. In addition to reducing the number of weeds, these authors also obtained a significant reduction in weed root length [37]. Also, *Bacillus* spp. used in wheat cultivation influenced the reduction in weeds [18]. Some rhizosphere bacteria colonize the roots of weeds and can inhibit their growth and development [1,38]. The main mechanism of biocontrol of weeds by bacteria suggested by many authors [1,18,20,39] is the production of phytotoxins, phytohormones (indoleacetic acid (IAA) and δ-aminolevulinic acid (ALA)), and antibiotics (blasticidin, taxtomin, hydantocidin, and methoxyhygromycin). In addition, as reported by Abbas et al. [40], some bacteria produce hydrogen cyanide (HCN), which inhibits weed growth by blocking many enzymes involved in the normal metabolic pathway. What is very significant in the previously mentioned field research by other authors is that, in addition to reducing weeds, the positive effect of bacterial formulation on the condition and yield of the main crop was revealed. So, an indirect method of reducing weed infestation may be to improve the growth of main crops as a result of bacterial formulations [10]. The promotion of plant growth as a result of PGPR applications is most often attributed to the synthesis of phytohormones, enzymes, mineral solubilization, and biological nitrogen fixation [41–44]. Thanks to this, among other things, there are changes in the architecture of plant roots, resulting in greater uptake of nutrients and water from the soil [45]. Thus, main plants become more competitive with weeds, limiting their access to resources [46]. However, some of the available results of field research found no effect of using bacterial formulation on crop weed infestation [31]. The reason for obtaining different results may be the species composition of the weeds present. Weissmann et al. [47] revealed a differential effect of *Serratia plymuthica* on the occurrence of a number of weeds, including *Chenopodium album*, *Stellaria media*, *Polygonum convolvulus*, and *Galeopsis speciosa*. In turn, Li and Kremer [48] demonstrated that *Pseudomonas fluorescens* inhibits the growth of *Ipomea* spp. and *Convolvulus arvensis* in wheat crops. Similarly, according to Zermane et al. [49], *Pseudomonas fluorescens* shows potential in controlling *Orobanche crenata* and *Orobanche foetida*. On the other hand, Dahiya et al. [18] demonstrated growth inhibition of *Avena fatua* L. in wheat crops under the application of *Bacillus spp*. This finding is also supported by the results of our own research, which revealed differential effects of the used consortia containing different bacteria on individual weed species. The consortium containing *Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Pseudomonas fluorescences* resulted in a more effective reduction of *Sinapis arvensis* and *Tripleurospermum inodorum*. In contrast, a consortium containing the bacteria *Bacillus meganterium* var. *Phosphaticum* and *Artrobacter agilis* more effectively reduced *Elymus repens*. In contrast, both bacterial consortia reduced the occurrence of *Chenopodium album* to a comparable degree.

Weed suppression is one of the key functions of introducing LM into the crop [28]. Our own research has proven a possible significant reduction in the weed infestation of organic barley crops when grown with LM. An analogous relationship in other crops has also been demonstrated by other authors [31,50,51]. The explanation for the reduction in weed incidence in crops with LM may be several mechanisms, but the most significant one is competition for light [52]. It is really important because nearly 50% of annual weeds require sufficient light to germinate [53]. Competition is especially important at the early stages of development, so rapid growth of LM can guarantee greater suppression of weeds [54]. Research conducted by Shili-Touzi [55] on wheat crops with alfalfa LM demonstrated that they absorb 20% more photosynthetic radiation during the initial growing periods compared to crops without LM. Moreover, the study found that maximum light absorption of more than 90% is reached at the BBCH 32 stage about 20 days earlier than in pure seeded wheat crops. This thus proves that LMs can help suppress weeds in the main crop throughout the growing season [56], thereby limiting the development of already germinated weeds as well as reducing weed seed production [54]. Mechanisms affecting weed reduction in LM crops also include competition for other resources such as water or soil nutrients [56]. Competition for water, like for light, is particularly important at the beginning of crop management, when both LM, weeds, and main crops require moisture inputs. Crops, unlike weeds, tend to have larger reserves in their seeds, so they can tolerate soil nutrient limitations better than weeds [28]. Thus, fast-growing LMs can initially reduce soil water availability without adversely affecting main plants while also limiting the ability of weeds to germinate. However, in the available literature, information on changes in soil water availability as a result of cultivation with LM is inconclusive [57,58]. Nevertheless, regardless, changes in water availability can have a much greater influence on shallow-rooted weeds than on deeper-rooted main crops [56]. In addition, very important competitive processes to obtain resources are dependent on each other, so a reduction in one will result in less efficient use of the others, as a result of which weed emergence and development will be reduced [52]. LM can also suppress weeds indirectly through allelopathy. However, this effect can vary between weed species. Research by Walters and Young [59] demonstrated improved control of *Amaranthus retroflexus* L. and no effect for *Digitaria ischaemum* control as a result of the introduction of LM into the main crop of winter rye. Also, in our own research, the use of LM for spring barley revealed differential effects on the dominant weed species. In our own research, the type of LM also had varying effects on both the reduction in total weeds and individual weed species. More effective control of the total number and weight of weeds was achieved with Italian ryegrass than with red clover. In general, an intermediate effect was obtained with the LM mixture. Analogous relationships were also obtained by other authors in their studies. Research conducted by Marcinkevičienė et al. [31] demonstrated a slight reduction in weeds in the main crop with the application of clover LM. On the other hand, in a study conducted by Sjursen et al. [30], a significantly greater reduction in weeds was found after the application of Italian ryegrass LM and a mixture of Italian ryegrass and red clover compared to red clover alone. Baraibar et al. [60] also found better weed suppression by grasses compared to legumes. This may be due to the rate of emergence and development of different types of LM [25]. Additionally, different plant species produce different levels of biomass, and many studies have revealed a strong negative correlation between LM biomass produced and weed biomass [61,62]. In addition, numerous studies [63–65] have proven the positive effect of PGPR on the development and morphological characteristics of grass and clover. Thus, the combination of the application of LM and PGPR in weed control may contribute to a more intensive development of LM and thus a stronger suppression of weeds. Our study demonstrated a different relationship between the occurrence of *Elymus repens* and other weed species. Presumably, the increased occurrence of this weed species on objects where Italian ryegrass was included in the LM compared to the other objects may have been due to the contamination of Italian ryegrass seeds with *Elymus repens* seeds.

In our own research, higher precipitation during the growing season resulted in higher weed pressure on crops. Also, in studies by other authors, higher weed pressure was recorded during growing seasons with a favorable distribution of precipitation and temperatures [66–68]. The greater occurrence of weeds under such conditions may be due to the increased availability of moisture in the soil, and thus, competition for this resource was lower. However, according to Ziska et al. [69], at higher temperatures and increased drought, weeds may become more competitive with crops. Climatic changes and varying conditions across growing seasons have a greater influence on weed species composition [6]. Thus, it can be assumed that under changing growing season conditions, some weed species that are more adapted, for example, to moisture deficits, can replace others that are sensitive to the conditions, filling a kind of gap. For example, according to Hanzlik and Gerowitt [70], wetter and milder winters will increase the survival rates of some annual weeds. Nevertheless, the proposed organic barley management techniques guaranteed a reduction in weed infestation regardless of growing season conditions.

5. Conclusions

It should be noted that frequent use of the same chemicals contributes to the development of segregated resistance mechanisms in plants. In addition, many studies have proven that the active substances contained in herbicides are toxic to the environment and to human and animal health.

Their negative impact on ecosystems, as well as the legislation being introduced to regulate the use and reduce the use of chemical pesticides (Integral Plant Protection and Sustainable Agriculture Strategy), are forcing the development of a new way to control weeds. Based on research, microbial formulations, which are developed on the basis of living organisms, among others, are the ideal solution. High specificity and selectivity, low production costs, and harmlessness to the environment and humans make biological plant protection products a safe alternative to chemical pesticides.

A good solution to increase the effectiveness of microorganism-based formulations seems to be their compilation with auxiliaries or plants that enhance and accelerate the action of herbicides. This is possible, among other things, through the use of living mulches, whose effectiveness can be based on the phenomenon of allelopathy. The impact of suitable living mulches involving the release of phytoncides (phytoncides, secondary metabolites) into the environment and competition for micronutrients and macronutrients at the same time can contribute to the reduction in weeds, which also needs to be taken into account and proven in future scientific research.

Testing in a field experiment of organic farming management techniques for spring barley demonstrated positive effects on reducing fresh and dry weed mass, as well as the number and species composition of weeds. The best results were obtained with a bacterial consortium containing *Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Pseudomonas fluorescences* in compilation with the LM of Italian ryegrass. However, due to the possibility of the undesirable introduction of *Elymus repens* into the cultivated area, the use of other LMs, including LMs in the form of plant mixtures, which also demonstrated positive effects, should also be considered. It is important that the application of the presented management techniques was effective, regardless of the growing season conditions. Thus, the use of bacterial consortia containing *Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Pseudomonas fluorescences*, along with the LM of Italian ryegrass or mixtures of red clover + Italian ryegrass can be successfully introduced on organic farms and a possible direction for sustainable agriculture.

Nevertheless, the greatest challenge in the development of biological pesticides seems to be strengthening public awareness and belief in the effectiveness and safety of their use.

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