Effects of Plant Growth Promoting Bacteria on chlorophyll a fluorescence parameters of Lolium perenne grown with microclover in drought conditions

Milena Truba

milena.truba@uws.edu.pl

University of Siedlce

Jakub Dobrzyński

Institute of Technology and Life Sciences—National Research Institute

Jacek Sosnowski

University of Siedlce

Research Article

Keywords:

Posted Date: May 29th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-6520497/v1

Additional Declarations: No competing interests reported.

Abstract

Background

Turf surface is a dominant element of green areas located within rural buildings or in cities. In recent years, mainly lawn varieties of grasses have been used to plant green areas, but microclover (*Trifolium repe*ns L.) has been increasingly popular. In addition, microclover can be effective in reducing irrigation costs. Drought is one of the main factors inhibiting the growth and development of plants. In modern agriculture some bacteria are used to promote plant growth and development ie. Plant growth promoting rhizobacteria (PGPR) increase systemic resistance of plants to biotic and abiotic stress. Chlorophyll fluorescence is used to measure the physiological condition and stress level of plants. Fluorescence analysis at O-J-I-P points (OJIP test) is a method used for the examination of plant vitality.

Results

The presence of microclover in the mixture contributed to the improvement of many chlorophyll a fluorescence parameters of Lolium perenne. Statistically, the maximum fluorescence (Fm), the maximum photochemical efficiency of PSII (Fv/Fm), and the maximum water-splitting efficiency on the donor side of PSII (Fv/Fo) increased significantly. The number of active reaction centers also increased, which translated into better energy flux absorbed by one reaction center (ABS/RC). In addition, there were upward tendencies of such parameters as the area above the induction curve (Area) and the density of reaction centers (RC/CSo). The use of Plant Growth Promoting Bacteria increased the maximum chlorophyll a fluorescence (Fm), the area above the induction curve (Area), and the density of reaction centers (RC/CSo).

Conclusion

Due to the above effects, microclover can be recommended to be used in lawn mixtures. The positive effect of the plant-promoting PGPB microbial vaccine on the Lolium perenne lawn variety suggests that more studies should be conducted on other plant species subjected to other kinds of abiotic stress. Growing plants exposed to drought stress with the substrate moisture level of 40% FWC did not affect the photosynthesis process. The parameter most sensitive to low substrate moisture was the rate of electron transport through one active reaction center (ETo/RC). Therefore, the ETo/RC parameter should be recommended for further research on drought stress.

Background

Drought is one of the main factors inhibiting the growth and development of plants [1]. Despite numerous studies on its mechanism, drought stress impact on plants is relevant as a topic and still frequently discussed [2-7]. A lack of soil moisture significantly inhibits photosynthesis, preventing the effective use of light energy, which in effect is dissipated as fluorescence or heat; these changes occur before the plant shows visible signs of turgor loss. That is why to measure drought stress, methods of chlorophyll a fluorescence determination have been used [8].

In modern agriculture some bacteria are used to promote plant growth and development. According to Furtak [9], the effects of using PGPB (plant growth-promoting bacteria) containing *Bacillus* spp. bacteria include greater resistance to long-term stress, accelerated seed germination, and the improved growth and extent of the root system. Furthermore, Consentino et al. [10] argue that PGPB are among the effective products reducing the use of mineral fertilizers. Those bacteria stimulate plant growth by producing various biologically active substances, such as phytohormones (IAA, gibberellins and cytokinins) and enzymes (ACC deaminase). Some of their enzymes are involved in biomass breakdown (cellulase, ligninase, and chitinase), in fixing atmospheric nitrogen (nitrogenase), and dissolving forms of phosphorus unavailable to plants [11, 12]. *Bacillus* bacteria are also included into the PGPR (plant growth promoting rhizobacteria) group, not only because they positively affect plant growth by producing hormones, but also because they increase the content of available nutrients in the soil and produce 1-aminocyclopropane-1-carboxylate deaminase to reduce ethylene concentration in plant tissues [13]. PGPR increase systemic resistance of plants to biotic and abiotic stress [14]. Bacillus strains are ubiquitous and can survive biotic and abiotic stress for long periods of time, forming spores in vivo. Some species of *Bacillus* have been successfully used as safe biological fertilizers and pesticides, due to their high survivability and as the most prominent rhizobacteria [15].

Chlorophyll fluorescence is used to measure the physiological condition and stress level of plants. Fluorescence analysis at O-J-I-P points (OJIP test) is a method used for the examination of plant vitality [16]. Developed by Strasser et al. [16], the test is applied to analyze photosynthetic apparatus condition during specific types of stress. It is used to determine the impact of stress on specific factors affecting the absorption of light and its conversion into biochemical energy. During the test, within the first second of illumination of the plant sample, previously kept in the dark, chlorophyll a fluorescence increases from the initial value (Fo) to the maximum (Fm) one [17, 18]. The results of the test, presented as a curve on a logarithmic scale, allow determining changes in the flow of excited electrons through the photosynthetic

apparatus. The O step in the curve represents an increase in chlorophyll a fluorescence emission from the initial level and reflects the gradual accumulation of QA, i.e. the primary quinone electron acceptor of Photosystem II (PSII) in its reduced form. The O–J phase of the curve represents the reduction of the acceptor side of PSII [2]. The J–I phase represents the process of the reduction of the plastoquinone (PQ) pool. The I-P phase, on the other hand, is the slowest and involves the transfer of electrons through Photosystem I (PSI) and is attributed to the reduction of the acceptor side of PSI [19].

The chlorophyll fluorescence induction curve and the maximum photochemical yield (Fv/Fm) are used as leading parameters detecting stress of various origins, affecting PSII [20, 21]. Another parameter is the maximum water-splitting efficiency (Fv/Fo), which, unlike Fv/Fm, is not an indicator of carbon assimilation but allows for faster detection of stress in the plant. In addition, the Fv/Fo ratio is used to calculate OJIP parameters. These parameters describe the energy fluxes inside and around the reaction center (RC) of PSII, i.e., specific energy fluxes per active reaction center (RC) and phenomenological energy fluxes per excited cross section (CS) of the sample [4, 22]. According to Olsen et al. [21], the absorption components (ABS/RC – energy absorbed per reaction center), trapped energy (TRo/RC – trapped energy flux per reaction center), and electron transport (ETo/RC- electron transport flux per reaction center) should be studied to analyze the location of interference within the photosynthetic apparatus. Pollastrini et al. [23] found that chlorophyll a fluorescence parameters were useful for analyzing plant responses to a variety of environmental stress factors and for phenotyping. According to Baker [24], drought stress can alter the steps of the OJIP test and reduce the intensity of fluorescence at the JIP phase. In negative environmental conditions, on the basis of the chlorophyll a fluorescence technique, it is possible to measure plant vigor, closely related to the functioning of PSII [25]. Additionally, the impairment of the photosynthetic activity affects CO2 fixation [26].

Turf surface is a dominant element of green areas located within rural buildings or in cities. Promoting biodiversity, green areas used for recreation play an underestimated role in the functioning of villages and cities. Additionally, as natural retention reservoirs they soak up rainwater. However, because of low soil moisture or a lack of irrigation, green areas often fail to fulfill their aesthetic function. In the literature there are reports on how drought can be mitigated by microbiological consortia and by using synthetic or natural water absorbents [27-30]. In recent years, mainly lawn varieties of grasses have been used to plant green areas, but microclover (*Trifolium repens L.)* has been increasingly popular. According to the literature, in a mixture with grass it fixes atmospheric nitrogen, introducing it to the soil through mineralization, which has a positive effect on the functional and aesthetical value of the lawn [31]. In addition, according to the research by Saeedipooya et al. [32], as a short plant, microclover can be effective in reducing irrigation costs.

The present paper examines the effect of PGPB and microclover on minimizing the current problem of drought and insufficient irrigation of green areas. In the research, the following research hypotheses were proposed:

1. Microclover grown in lawn mixtures will have a positive effect on mitigating the effects of drought.

2. The PGPB bacterial strain will minimize drought stress in *Lolium perenne* grown with microclover and in pure stand.

Material and methods

Plant growth conditions and experimental design

The pot experiment was conducted from the beginning of November 2023 to mid-January 2024 in a phytotron, ensuring constant growing conditions. The air temperature in the light and dark-adapted stage was 22°C, with the light intensity of 200 µmol m^{-2} s^{-1} (obtained with high-pressure sodium lamps) and the photoperiod of 16 h in light. The 1.5L pots were filled with soil, which, according to the Bouyoucos–Casagrande aerometric method modified by Prószyński, was light clay [33].

The following plants were used in the experiment: *Lolium perenne* 2N lawn Boxer, seeds produced by the Granum Seed Company, Wodzierady, Poland, as certified seed material; *Trifolium repens* L. (microclover) cv. Pipolina seeds purchased from Eco Deco, Kraków, Poland as certified seed material. The seeds of *Lolium perenne* were sown into some pots, with the seeding rate of 36 g m^{-2} (Lp1). Other plots were planted with *Lolium perenne* (34.9 g m^{-2}) together with microclover (1.1 g m^{-2}), with the latter constituting a 3% share in the mixture (Lp2). The above Lp1 and Lp2 plots were the main research factor. The second factor was drought stress, achieved by maintaining the soil moisture levels of 40% FWC (Field Water Capacity), while FWC in other pots was 60%. The third research factor was the strain of *Bacillus* spp. N188 producing ACC deaminase – referred to as PGPB in this paper. PGPB were not applied to control pots. The experiment was conducted in four replications. Combinations of experimental factors are presented in Table 1.

Table 1 Methodological data

Type of crop	Field Water Capacity			
	40% FWC		60% FWC	
	Soil treatment			
	Control	PGPB	Control	PGPB
Lp1	Lp1	Lp1	Lp1	Lp1
*Lolium perenne*100%	40% FWC	40%FWC/PGPB	60% FWC	60%FWC/PGPB
Lp2	Lp2	Lp2	Lp2	Lp2
Lolium perenne 97%	40% FWC	40%FWC/PGPB	60% FWC	60%FWC/PGPB
Microclover 3%				

Microbiological material

When plants germinated, the Bacillus spp. strain producing ACC deaminase (PGPB) was applied to the soil with a pipette, in the amount of 5mL per pot. The N188 strain was isolated from the soil by the Institute of Technology and Life Sciences - National Research Institute in Falenty (Poland). Approximately 100 bacterial strains were isolated from various soils, including both rhizosphere and bulk soil. Bacterial colonies were selected and passaged until pure strains were obtained. Then the strains were inoculated, according to Patil et al. [34], onto ACC minimal media with bromothymol blue (BTB). The N188 strain was selected, with phenol red as a marker, and grown at 30°C for 24 hours with shaking at 150 rpm in 5 mL of Lysogeny Broth (LB). Then, the strain was inoculated onto Dworkin and Foster medium amended with 3 mM ACC (instead of $(NH_4)_2SO_4$) as the sole nitrogen source [35,36]. After incubation, the bacterial culture was centrifuged at 6000 rpm for 10 minutes. The resulting pellet was suspended in 1 ml of 0.1 M Tris-HCl buffer at pH 7.6, and then the process was continued according to the method described by Penrose and Glick [36]. ACC deaminase activity was calculated by measuring alpha-ketobutyric acid formed by the cleavage of ACC by the ACC deaminase enzyme. Then the selected bacterial strain was identified by sequencing the 16S rRNA gene. To amplify the 16S rRNA genes, universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') were used. The PCR (polymerase chain reaction) products were sequenced by the Sanger technique (NEXBIO, Lublin, Poland). The obtained sequences were assembled to contig (BioEdit) and compared with the sequences from GenBank, EMBL (European Molecular Biology Laboratory), using BLAST (the Basic Local Alignment Search Tool). The 16S rRNA gene sequences of the bacterial strain were deposited in GenBank under accession number PP500625.

Chlorophyll a fluorescence

After the sample was adapted in the dark, measurements on flag leaf blades were made with the OS30p+ fluorometer (plant stress meter), using a clip. Each measurement was preceded by 30 minutes of adaptation in the dark. Then OJIP measurements were made. As a result of the OJIP test measurement, the chlorophyll a fluorescence parameters presented in Table 2 were obtained.

Table 2 The description of fluorescence parameters (in relative value)

The data obtained in the OJIP test and other parameters calculated	
Abbreviation	Description
O	Fluorescence at 20 μs
K	Fluorescence at 300 μs
J	Fluorescence at 2 ms
I	Fluorescence at 30 ms
P = Fm	Fluorescence at 3s (with maximum fluorescence measured)
Fo	Minimum fluorescence
Fv/Fm	Maximum photochemical efficiency of PSII
Fv/Fo	Maximum water-splitting efficiency on the donor side of PSII
Area	The area between the OJIP curve and Fm
ABS/RC	Energy absorbed by one active reaction center
TRo/RC	Energy flux trapped by one active reaction center (RC)
ETo/RC	Electron transport flux per one active reaction center (RC)
DIo/CS	Thermal dissipation of excitation energy by PSII of a photosynthetic sample
ETo/CSo	Electron transport flux per cross section (CS) of a sample
RC/CSo	Density of reaction centers (QA reducing PSII reaction centers)

Statistics

The results of the research were processed using the Statistica 13 program (TIBCO Soft-ware Inc., PaloAlto, CA, USA). The data were analyzed at the significance level of $p<0.05$. The differences between means were assessed with ANOVA and Tukey's HSD test (Honest Significant Difference). In the tables the significance of the differences between means is indicated by lowercase and uppercase letters.

Results and discussion

Chlorophyll a fluorescence induction curve (OJIP curve)

Chlorophyll a fluorescence induction curves presented in Figure 1 illustrate the plants' physiological condition for each experimental unit. The lowest chlorophyll a fluorescence was recorded in control *Lolium perenne* grown in pure stand (Lp1) on the soil with 60% moisture.

On the other hand, the highest values were noted for the grass grown in a mixture with microclover (Lp2) on the soil with the 60% moisture. The fact that curves for *Lolium perenne* grown together with microclover on the soil with 60% moisture (Lp2/60%) were higher than the others might be attributed to the presence of the legume plant in the mixture. Due to its ability to fix atmospheric nitrogen, microclover introduced its additional amount into the soil. In effect plants in the mixture were nourished better and chlorophyll a fluorescence reached greater values. Similarly, Kalaji et al. [37] confirmed that the supply of plants with nitrogen resulted in an increase in fluorescence.

The induction curve of *Lolium perenne* grown in pots with 40% FWC did not change so dynamically. However, there were clear differences between the Lp1 and Lp2 curves, with the latter reaching greater values than the former, both for 40% WFC and 60% WFC. The absence of a distinct K-point on all chlorophyll fluorescence induction curves indicated that the photosynthesis process of plants grown on both 40 and 60% FWC soil was not disturbed. According to the literature, the K-peak can be used as a potential indicator of physiological instability under drought stress even before the appearance of visible signs on plants [5, 18]. The K-point on the chlorophyll a fluorescence curve is caused by the inactivation of the oxygen-releasing complex or by the inhibition of electron transport on the donor and acceptor side of PSII [18]. The multivariate analysis of variance showed no statistically significant effect of either FWC or PGPB or their interaction on chlorophyll fluorescence (Table 3). On the other hand, the effects of the crop type and its interaction with PGPB or FWC were statistically significant. Similarly, statistically significant differences were also noted in the triple interaction of all factors on the O-J fragment of the induction curve.

Table 3 Chlorophyll fluorescence assessed by the OJIP test

	Crop type	FWC	PGPB	Crop type x PGPB	%FWC x PGPB	Crop type x %FWC	Crop type x PGPB x %FWC
O	44.25*	ns	ns	74.17*	ns	88.50*	89.00*
K	59.75*	ns	ns	91.16*	ns	113.17*	112.67*
J	78.17*	ns	ns	117.34*	ns	87.00*	147.34*
I	165.88*	ns	ns	221.97*	ns	267.33*	ns
P	193.12*	ns	ns	ns	ns	312.13*	ns

HSD values from three-way analysis of variance for the effects of crop type, i.e. Lp1 and Lp2; field water capacity (FWC) i.e. 40% and 60%; treatment, i.e. control and PGPB on OJIP parameters. *p≤0.05; ns p>0.05

The results indicated that the application of PGPB significantly increased chlorophyll a fluorescence both for plants grown in Lp1 and Lp2 pots, with Lp2 values being visibly greater (Figure 2a). Chlorophyll a fluorescence for Lp1 plants was greater when soil FWC was 40%, while in Lp2 pots it was greater at 60% FWC (Fig. 2b). It can be assumed that optimal moisture content and the presence of microclover in the mixture contributed to the intensification of the chlorophyll a fluorescence process. According to Digrado et al. [38], low soil moisture had a small effect on PSII performance, but it increased the PSII sensitivity of *Lolium perenne* to heat stress.

Chlorophyll a fluorescence parameters assessed by the OJIP test

Compared to control, PGPB increased the minimum fluorescence (Fo) to a large extent (Table 4). Additionally, a 9% increase in Fo indicated an interaction between the type of crop and PGPB. The literature reports that plants react with a chlorophyll a fluorescence increase after the application of plant supporting products [39, 40]. In the present experiment no significant differences were found between the minimum fluorescence (Fo) values for *Lolium perenne* grown in pure stand and in the mixture. There was a slight difference between pots with different FWC values, for non-irrigated pots this value was on average 1.6% greater. The Fo value was measured with plant receptors open in the dark-adapted state. The measurements were taken in a faint red light so as not to excite the photosynthetic process. According to the literature, greater values indicate lower efficiency of excitation energy transfer between pigment molecules in the PSII energy antenna [41]. On the other hand, photoprotection or an increase in CO_2 fixation can contribute to a Fo decrease.

Table 4 Minimum chlorophyll a fluorescence of dark-adapted state (Fo)

Type of crop	Moisture	Soil treatment		Mean
		Control	PGPB	
Lp1	40% FWC	319.00±1.00Bb	389.33±8.08Aa	354.17A
	60% FWC	308.33±5.51Bb	340.33±5.51Ba	324.33 A
Lp2	40% FWC	312.33±14.15Bb	342.33±5.69Ba	327.33 A
	60% FWC	347.67±10.41Aa	344.00±3.61Ba	345.83 A
Mean for moisture				
40% FWC		315.67Ab	365.83Aa	340.75 A
60% FWC		328.00Aa	340.17Aa	335.08 A
Mean for type of crop				
Lp1		313.67From	364.83Aa	339.25A
Lp2		330.00Aa	343.17Aa	336.58A
Mean		321.83 b	354.00 a	

The means in columns marked with the same uppercase letters do not differ significantly. The means in lines marked with the same lowercase letters do not differ significantly. Abbreviations: Lp1 – *Lolium perenne* in pure stand; LP2 - *Lolium perenne* in a mixture with microclover; 40% FWC – 40% field water capacity; 60% FWC – 60% field water capacity; PGPB – Plant Growth Promoting Bacteria

The maximum fluorescence values varied (Table 5). It was observed that *Lolium perenne* plants growing with the leguminous plant (Lp2) had a much greater Fm value than *Lolium perenne* in pure stand (Lp1). According to Kalaji et al. [37], even small doses of nitrogen increased Fm in spring barley. Kalaji and Łoboda [42] stated that nitrogen reduction in relation to the control sample indicated the occurrence of stress, as a result of which not all electron acceptors in PSII were completely reduced.

Table 5 Maximum chlorophyll a fluorescence (Fm)

Type of crop	Moisture	Soil treatment		Mean
		Control	PGPB	
Lp1	40% FWC	991.33±240.58 Aa	1090.7±129,68 Aa	1041.0AB
	60% FWC	902.33±241.47 Aa	916.00±135.52 Aa	909.17B
Lp2	40% FWC	1052.0±135.84 Aa	1178.0±175.45 Aa	1115.0AB
	60% FWC	1207.0±113.85 Aa	1235.7±137.17 Aa	1221.0A
Mean for moisture				
40% FWC		1021.7 Aa	1134.3 Aa	1078.0A
60% FWC		1054.7 Aa	1075.8 Aa	1065.3A
Mean for type of crop				
Lp1		946.83 Aa	1003.3 Aa	975.08B
Lp2		1129.5 Aa	1206.8 Aa	1168.2A
Mean		1038.2a	1105.1a	

Means in columns marked with the same uppercase letters do not differ significantly. Means in lines marked with the same lowercase letters do not differ significantly. Abbreviations: Lp1 – *Lolium perenne* in pure stand; LP2 - *Lolium perenne* in a mixture with microclover; 40% FWC – 40% field water capacity; 60% FWC – 60% field water capacity; PGPB – Plant Growth Promoting Bacteria

Wang et al. [43] observed that in a *Bothriochloa ischaemum* and *Lespedeza davurica* mixture, Fm increased with an increasing nitrogen dose. In the present experiment there was no significant effect of substrate moisture on Fm. There was a slight tendency for the PGPB plants to have a 6% greater Fm value compared to control. It is possible that there was an interaction between microclover plants and PGPB *Bacillus* microorganisms, which increased the Fm value in *Lolium perenne* growing together with the leguminous plant.

The Fv/Fm parameter varied within the experiment and ranged from 0.6 to 0.7 (Table 6). Its values differed significantly between *Lolium perenne* from Lp1 and Lp2 pots. *Lolium perenne* grown with microclover had about 9.5% greater value of the maximum photochemical efficiency of PSII.

Table 6 Maximum photochemical efficiency of PSII (Fv/Fm)

Type of crop	Moisture	Soil treatment		Mean
		Control	PGPB	
Lp1	40% FWC	0.665±0.080Aa	0.639±0.048 Aa	0.652A
	60% FWC	0.642±0.088 Aa	0.623±0.053 Aa	0.632A
Lp2	40% FWC	0.698±0.050 Aa	0.704±0.046 Aa	0.701A
	60% FWC	0.709±0.033 Aa	0.719±0.032 Aa	0.714A
Mean for moisture				
40% FWC		0.682Aa	0.671 Aa	0.677A
60% FWC		0.676 Aa	0.671 Aa	0.673A
Mean for type of crop				
Lp1		0.653 Aa	0.631 Aa	0.642B
Lp2		0.704 Aa	0.711 Aa	0.708A
Mean		0.679 a	0.671 a	

Means in columns marked with the same uppercase letters do not differ significantly. Means in lines marked with the same lowercase letters do not differ significantly. Abbreviations: Lp1 – *Lolium perenne* in pure stand; LP2 - *Lolium perenne* in a mixture with microclover; 40% FWC – 40% field water capacity; 60% FWC – 60% field water capacity; PGPB – Plant Growth Promoting Bacteria

According to Angelini et al. [44], in optimal plant growth conditions, its value should be about 0.85 relative units, and its decrease indicates the occurrence of stress. In the present research, the parameter did not change with the change in soil moisture, which proves it is not sensitive to drought stress. Kalaji et al. [22] also reported that some kinds of stress, e.g. drought, did not affect Fv/Fm value. Digrado et al. [38] obtained the highest Fv/Fm in *Lolium perenne* in autumn, with average values of approx. 0.75, which indicated a high PSII efficiency. In summer, on the other hand, values as low as 0.15 were noted. Lower Fv/Fm values in summer indicated a reduced photochemical efficiency of PSII and stronger energy dissipation, but the plant returned to its full photochemical activity after the stress subsided.

The greatest value of the maximum efficiency of water splitting on the PSII donor side was noted for *Lolium perenne* plants grown in the mixture (Table 7). According to Aazami et al. [45], plant stress caused by adverse conditions can lead to temporary photoinhibition or damage of photosynthetic organs, resulting in a decrease in chlorophyll fluorescence parameters. This was confirmed by Li et al. [46], who observed that drought stress combined with high temperature resulted in Fv/Fo values falling sharply. Olsen et al. [21] observed that when Fo increased in stressed plants, the Fv/Fo ratio decreased.

Table 7 Maximum water-splitting efficiency on the PSII donor side (Fv/Fo)

Type of crop	Moisture	Soil treatment		Mean
		Control	PGPB	
Lp1	40% FWC	2.108±0.761Aa	1.806±0.388 Aa	1.957A
	60% FWC	1.924±0.774 Aa	1.690±0.383 Aa	1.807A
Lp2	40% FWC	2.385±0.597 Aa	2.446±0.566 Aa	2.416A
	60% FWC	2.479±0.423 Aa	2.594±0.431 Aa	2.536A
Mean for moisture				
40% FWC		2.247 Aa	2.126 Aa	2.186A
60% FWC		2.201 Aa	2.142 Aa	2.172A
Mean for type of crop				
Lp1		2.016 Aa	1.748 Aa	1.882B
Lp2		2.432 Aa	2.520 Aa	2.476A
Mean		2.224a	2.134a	

Means in columns marked with the same uppercase letters do not differ significantly. Means in lines marked with the same lowercase letters do not differ significantly. Abbreviations: Lp1 – *Lolium perenne* in pure stand; LP2 - *Lolium perenne* in a mixture with microclover; 40% FWC – 40% field water capacity; 60% FWC – 60% field water capacity; PGPB – Plant Growth Promoting Bacteria

Statistical analysis of the Area parameter did not show any significant differences between different experimental units, but some tendencies were notable (Table 8). The area above the Lp2 *Lolium perenne* curves was on average 25% greater, which meant that for those units the number of electron acceptors of photosynthetic electron transport chain (PETC) was also greater. A visible difference was also noticed between the average values of the Area parameter for pots with and without PGPB. Plants with PGPB had a 13% greater number of PETC electron acceptors. Soil moisture content did not significantly affect the Area parameter, and the difference in the average values between 40%FWC and 60%FWC was about 5%. Schansker et al. [47] argue that there is a relationship between the area over the OJIP transients and the number of electrons transported through the PETC before the Fm value is reached. Strasser et al. [16] and Khan et al. [48] stated that this area was proportional to the number of PETC electron acceptors.

Table 8 Area over OJIP Induction Curve

Type of crop	Moisture	Soil treatment		Mean
		Control	PGPB	
Lp1	40% FWC	8379.3±4671.4Aa	9640.7±3429.5 Aa	9010.0 A
	60% FWC	7429.3±2982.3 Aa	7401.0±2954.6 Aa	7415.3 A
Lp2	40% FWC	9210.7±2650.5 Aa	10545.0±3978.4 Aa	9879.0 A
	60% FWC	9700.0±1721.8 Aa	11603.0±2756.9 Aa	10652.0 A
Mean for moisture				
40% FWC		8795.0 Aa	10094.0 Aa	9444.5A
60% FWC		8564.8 Aa	9502.0 Aa	9033.4A
Mean for type of crop				
Lp1		7904.5 Aa	8520.8 Aa	8212.7A
Lp2		9455.3 Aa	11075.0 Aa	10265.0A
Mean		8679.9a	9798.0a	

Means in columns marked with the same uppercase letters do not differ significantly. Means in lines marked with the same lowercase letters do not differ significantly. Abbreviations: Lp1 – *Lolium perenne* in pure stand; LP2 - *Lolium perenne* in a mixture with microclover; 40% FWC – 40% field water capacity; 60% FWC – 60% field water capacity; PGPB – Plant Growth Promoting Bacteria

Figures 3, 4, and 5 present the differences in percentage between the effects of experimental factor. The pot with *Lolium perenne* grown in pure stand at optimal field water capacity and without the microbiological vaccine (Lp1/60% FWC) was used as a reference value (100%). Figure 3 presents the effects of experimental factors on Fo, Fm, Fv/Fv, Fv/Fm, and Area parameters. They took greater values for Lp2 pots than for Lp1 ones, which meant that microclover affected the growth and physiological functions of *Lolium perenne*. A beneficial effect of PGPB was also recorded for pots with plants in drought stress (40% FWC/PGPB), where Fm and Area increased on average by approx. 25% compared to the reference value (Lp1/60% FWC).

Specific energy flux per one active reaction center (RC) of PSII

The energy absorbed by one active reaction center (ABS/RC) was significantly greater, by 12%, in *Lolium perenne* plants in pure stand than in *Lolium perenne* grown with microclover (Table 9). No statistically significant differences were noted between the effects of the other experimental factors, but some trends were observed. A 5% increase in the flux of energy absorbed by one active reaction center was noted for 40% FWC pots compared to 60% FWC ones.

Table 9 Energy absorbed by one active reaction center (ABS/RC)

Type of crop	Moisture	Soil treatment		Mean
		Control	PGPB	
Lp1	40% FWC	2.828±0.569 Aa	2.5153±0.343 Aa	2.6718A
	60% FWC	2.463±0.405 Aa	2.6107±0.404 Aa	2.5368A
Lp2	40% FWC	2.2350±0.206 Aa	2.3920±0.290 Aa	2.3135A
	60% FWC	2.2937±0.210 Aa	2.2523±0.395 Aa	2.2730A
Mean for moisture				
40% FWC		2.5317 Aa	2.4537 Aa	2.4927A
60% FWC		2.3783 Aa	2.4315 Aa	2.4049A
Mean for type of crop				
Lp1		2.6457 Aa	2.5630 Aa	2.6043A
Lp2		2.2643 Aa	2.3222 Aa	2.2933B
Mean		2.4550a	2.4426a	

Means in columns marked with the same uppercase letters do not differ significantly. Means in lines marked with the same lowercase letters do not differ significantly. Abbreviations: Lp1 – *Lolium perenne* in pure stand; LP2 - *Lolium perenne* in a mixture with microclover; 40% FWC – 40% field water capacity; 60% FWC – 60% field water capacity; PGPB – Plant Growth Promoting Bacteria

Monneveux et al. [49] noted an increase in the ABS/RC parameter in plants during drought stress and argued that that indicated that some reaction centers were inactive and the yield per reaction center increased. The ABS/RC parameter also reflects the effective antenna size of the active reaction centers; as the parameter increases, the antenna size of the active reaction centers decreases [50]. Therefore, in the present studies, the antenna of the active reaction centers was larger in the case of *Lolium perenne* plants on soil with the substrate moisture of 60% FWC and in those grown with microclover.

The values of the energy flux trapped by one active reaction center (TRo/RC) did not vary across research factors, ranging on average from 1.59 to 1.67 (Table 10). Using *P. scutellarioides* plants, Meng et al. [18] observed that the TRo/RC parameter increased in response to drought stress only for the first 10 days, and then it began to decrease. The authors concluded that to resist drought stress and to maintain their growth, plants inhibited electron transport and reduced the PSII photochemical activity at an earlier stage of stress.

Table 10 Energy flux trapped by one active reaction center (RC), at time 0 (TRo/RC)

Type of crop	Moisture	Soil treatment		Mean
		Control	PGPB	
Lp1	40% FWC	1.7845±0.130 Aa	1.5605±0.176 Aa	1.6725A
	60% FWC	1.6174±0.066 Aa	1.6582±0.154 Aa	1.6378A
Lp2	40% FWC	1.5821±0.074 Aa	1.7161±0.165 Aa	1.6491A
	60% FWC	1.6009±0.150 Aa	1.5758±0.182 Aa	1.5884A
Mean for moisture				
40% FWC		1.6833 Aa	1.6383 Aa	1.6608A
60% FWC		1.6092 Aa	1.6170 Aa	1.6131A
Mean for type of crop				
Lp1		1.7010 Aa	1.6093 Aa	1.6551A
Lp2		1.5915 Aa	1.6460 Aa	1.6187A
Mean		1.6462a	1.6277a	

Means in columns marked with the same uppercase letters do not differ significantly. Means in lines marked with the same lowercase letters do not differ significantly. Abbreviations: Lp1 – *Lolium perenne* in pure stand; LP2 - *Lolium perenne* in a mixture with microclover; 40% FWC – 40% field water capacity; 60% FWC – 60% field water capacity; PGPB – Plant Growth Promoting Bacteria

In the present experiment no statistically significant effect of experimental factors on the electron transport flux through one active reaction center (ETo/RC) was noted. However, this parameter was 6.5% greater for plants in drought stress, growing on substrate with 40% FWC (Table 11). According to the literature [51, 52], in waterlogged conditions plants reduce the ETo/RC parameter value because there are fewer active RCs and more QA reduction. The reoxidation of reduced QA through electron transport in the active RC is also reduced, indicating a decrease in ETo/RC.

Table 11 Rate of electron transport through one active reaction center, at time 0 (ETo/RC)

Type of crop	Moisture	Soil treatment		Mean
		Control	PGPB	
Lp1	40% FWC	6.4554±0.753 Aa	5.6524±0.745 Aa	6.0539A
	60% FWC	5.8205±0.869Aa	5.7484±0.518 Aa	5.7845A
Lp2	40% FWC	6.1488±0.241 Aa	6.6594±0.606 Aa	6.4041A
	60% FWC	5.6624±1.164 Aa	5.9708±0.09 Aa	5.8166A
Mean for moisture				
40% FWC		6.3021 Aa	6.1559 Aa	6.2290A
60% FWC		5.7415 Aa	5.8596 Aa	5.8006A
Mean for type of crop				
Lp1		6.1380 Aa	5.7004 Aa	5.9192A
Lp2		5.9056 Aa	6.3151 Aa	6.1103A
Mean		6.0218a	6.0078a	

Means in columns marked with the same uppercase letters do not differ significantly. Means in lines marked with the same lowercase letters do not differ significantly. Abbreviations: Lp1 – *Lolium perenne* in pure stand; LP2 - *Lolium perenne* in a mixture with microclover; 40% FWC – 40% field water capacity; 60% FWC – 60% field water capacity; PGPB – Plant Growth Promoting Bacteria

Figure 4 present the effects of experimental factors on ABS/RC, TRo/RC, and ETo/RC parameters. Their highest values were recorded for *Lolium perenne* in pure stand, not treated with PGBG and subjected to drought stress (Lp1/40% WFC). The values for the 40% FWC pots with PGPB were nearly the same as the reference value (Lp1/60% FWC). On the other hand, the lowest values of ABS/RC, TRo/RC, and ETo/RC parameters were recorded in *Lolium perenne* plants growing in a mixture with microclover, with substrate moisture content of 60% FWC and without PGPB (L2/60% FWC).

Fig. 4 The effect of experimental factors on ABS/RC, TRo/RC, and ETo/RC parameters. Abbreviations: Lp1 – *Lolium perenne* in pure stand; LP2 - *Lolium perenne* in a mixture with microclover; 40% FWC – 40% field water capacity; 60% FWC – 60% field water capacity; PGPB – Plant Growth Promoting Bacteria

Phenomenological energy fluxes per excited cross section of the photosynthetic sample (CS) Density of active reaction centers (RCs) of PSII

The results (Table 12, 13, 14) did not show any significant effects of the crop type, %FWC, or PGPB, or their double or triple interactions on the reaction center density (RC/CSo), electron transport by PSII (Eto/CSo), and thermal dissipation of excitation energy by PSII (DIo/CS). Despite the lack of statistically significant differences, many downward or upward tendencies in the above-mentioned parameters were observed. Thus, in the case of the RC/CSo parameter, the number of reaction center densities for *Lolium perenne* growing in the mixture with microclover was about 10% greater than for *Lolium perenne* in pure stand (Table 12). In addition, the use of PGPB also resulted in an increase in the RC/CSo parameter by approx. 9%.

Table 12 Density of plastoquinone QA reducing reaction centers (RC/CSo)

Type of crop	Moisture	Soil treatment		Mean
		Control	PGPB	
Lp1	40% FWC	112.26±24.31 Aa	136.96±18.80 Aa	124.61A
	60% FWC	131.74±20.71Aa	151.05±18.99 Aa	141.40A
Lp2	40% FWC	156.20±11.04 Aa	145.12±16.76 Aa	150.66A
	60% FWC	136.66±9.86 Aa	155.08±26.75 Aa	145.87A
Mean for moisture				
40% FWC		134.23 Aa	141.04 Aa	137.64A
60% FWC		134.20 Aa	153.07 Aa	143.63A
Mean for type of crop				
Lp1		122.00 Aa	144.01 Aa	133.00A
Lp2		146.43 Aa	150.10 Aa	148.27A
Mean		134.21a	147.05a	

Means in columns marked with the same uppercase letters do not differ significantly. Means in lines marked with the same lowercase letters do not differ significantly. Abbreviations: Lp1 – *Lolium perenne* in pure stand; LP2 - *Lolium perenne* in a mixture with microclover; 40% FWC – 40% field water capacity; 60% FWC – 60% field water capacity; PGPB – Plant Growth Promoting Bacteria

The results indicated that the electron transport per cross section of the photosynthetic sample (ETo/CSo) had similar values for both 40 and 60 % FWC. Minor differences were noted between the average ETo/CSo results for *Lolium perenne* growing in a mixtu*re* with microclover and in pure stand (Table 13). For the former the value was about 13% greater than for the latter. Similarly, the use of PGPB increased the electron transport flux by about 8%.

Table 13 Electron transport flux per cross section (ETo/CS)

Type of crop	Moisture	Soil treatment		Mean
		Control	PGPB	
Lp1	40% FWC	778.14±234.09 Aa	870.68±159.57 Aa	824.41A
	60% FWC	735.63±234.13 Aa	778.69±184.41 Aa	757.16A
Lp2	40% FWC	772.29±160.21 Aa	925.44±156.50 Aa	848.87A
	60% FWC	962.14±104.55 Aa	964.82±123.87 Aa	963.48A
Mean for moisture				
40% FWC		775.21 Aa	898.06 Aa	836.64A
60% FWC		848.88 Aa	871.76 Aa	860.32A
Mean for type of crop				
Lp1		756.88 Aa	824.69 Aa	790.78A
Lp2		867.21 Aa	945.13 Aa	906.17A
Mean		812.05a	884.91a	

Means in columns marked with the same uppercase letters do not differ significantly. Means in lines marked with the same lowercase letters do not differ significantly. Abbreviations: Lp1 – *Lolium perenne* in pure stand; LP2 - *Lolium perenne* in a mixture with microclover; 40% FWC – 40% field water capacity; 60% FWC – 60% field water capacity; PGPB – Plant Growth Promoting Bacteria

No major differences for the thermal dissipation of excitation energy by PSII of the photosynthetic sample (DIo/CS) between the experimental units were observed (Table 14).

Table 14 Thermal dissipation of excitation energy by PSII of a photosynthetic sample (DIo/CS)

Type of crop	Moisture	Soil treatment		Mean
		Control	PGPB	
Lp1	40% FWC	1.0442±0.44331 Aa	0.95530±0.22540 Aa	0.99970A
	60% FWC	0.84627±0.33953 Aa	0.95303±0.26371 Aa	0.89965A
Lp2	40% FWC	0.69317±0.14294 Aa	0.67697±0.21421 Aa	0.68507A
	60% FWC	0.65307±0.13287 Aa	0.67647±0.14323 Aa	0.66477A
Mean for moisture				
40% FWC		0.84862 Aa	0.81585 Aa	0.83223A
60% FWC		0.76972 Aa	0.81500 Aa	0.79236A
Mean for type of crop				
Lp1		0.94522 Aa	0.95413 Aa	0.94967A
Lp2		0.67312 Aa	0.67672 Aa	0.67492A
Mean		0.80917a	0.81542a	

Means in columns marked with the same uppercase letters do not differ significantly. Means in lines marked with the same lowercase letters do not differ significantly. Abbreviations: Lp1 – *Lolium perenne* in pure stand; LP2 - *Lolium perenne* in a mixture with microclover; 40% FWC – 40% field water capacity; 60% FWC – 60% field water capacity; PGPB – Plant Growth Promoting Bacteria

Figure 5 presents the effect of experimental factors on of RC/CSo, ETo/CSo, and DIo/CS parameters. The thermal dissipation of excitation energy by PSII of the sample (DIo/CS) was more beneficial for units with *Lolium perenne* grown in the mixture (20% lower than for Lp1). On the other hand, its values for *Lolium perenne* in pure stand were greater than for Lp2 plants, both on 40% and 60% FWC substrate or whether PGPB was used or not. Electron transport flux per cross section (ETo/CSo) was the greatest in *Lolium perenne* grown with microclover on substrate with optimal field water capacity (Lp2/60% FWC). Increased values of this parameter were also noted for the Lp2 and Lp1 plants grown on substrate with 40% FWC and with PGPB. Density of reaction centers (RC/CSo) was greater in *Lolium perenne* grown with microclover, with PGPB and 60% FWC (L2/60% FWC/PGPB).

The RC/CSo and ETo/CSo parameters are often used to determine damage to the PSII reaction center, caused by adverse environmental conditions [53]. However, as the results indicated, drought stress caused by the moisture level of 40% FWC did not significantly affect the functioning of the PSII and no damage occurred. According to Zhao et al. [6], with low soil water content (<29.7%), the PSII reaction center absorbed significantly more light energy, but the ability to capture and transmit it was significantly reduced; therefore, the accumulation of excess light energy eventually reduced photosynthetic efficiency, with a significant decrease in RC/CSo and ETo/CSo values. Chen et al. [54] and Zhang et al. [55] stated that this indicated that excessive excitation energy was stored at the reaction center, causing the electron transfer on the PSII acceptor side to be interrupted, with the photosynthetic apparatus further damaged. A tendency to decrease RC/CSo, ETo/CSo and increase DIo/CS as a result of subjecting the plant to drought stress was also noted by Contreras-Porcia et al. [56] and Olsen et. al. [21].

Conclusions

The presence of microclover in the mixture contributed to the improvement of many chlorophyll a fluorescence parameters of *Lolium perenne*. Statistically, the maximum fluorescence (Fm), the maximum photochemical efficiency of PSII (Fv/Fm), and the maximum water-splitting efficiency on the donor side of PSII (Fv/Fo) increased significantly. The number of active reaction centers also increased, which translated into better energy flux absorbed by one reaction center (ABS/RC). In addition, there were upward tendencies of such parameters as the area above the induction curve (Area) and the density of reaction centers (RC/CSo). Due to the above effects, microclover can be recommended to be used in lawn mixtures. The use of Plant Growth Promoting Bacteria increased the maximum chlorophyll a fluorescence (Fm), the area above the induction curve (Area), and the density of reaction centers (RC/CSo). The positive effect of the plant-promoting PGPB microbial vaccine on the *Lolium perenne* lawn variety suggests that more studies should be conducted on other plant species subjected to other kinds of abiotic stress. As the results indicated, growing plants exposed to drought stress with the substrate moisture level of 40% FWC did not affect the photosynthesis process. The parameter most sensitive to low substrate moisture was the rate of electron transport through one active reaction center (ETo/RC). Therefore, the ETo/RC parameter should be recommended for further research on drought stress.

Declarations

Abbreviations

Not applicable.

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

M.T. - study design; J.D. - preparation of microbiological formula; M.T. and J.S. - data collection; M.T. - statistical analysis, M.T. - data interpretation; M.T., J.D. and J.S. - manuscript preparation; M.T., J.D. and J.S. - literature search.

Acknowledgements

Not applicable.

Funding

This research was supported by Ministry of Science and Higher Education – Poland, Grant No. 161/23/B.

Ethics declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

References

1. Farooq M, Wahid A, Kabayashi N, Fujita D, Basra SMA. Plant drought stress: effects, mechanisms and management. In: Sustainable Agriculture, Edited by Lichtfouse E, Navarrete M, Debaeke P, Souchere V, Alberola C. Springer Publishers 2009: 153-188.
2. Oukarroum A, Madidi S El, Schansker G, Strasser RJ. Probing the responses of barley cultivars (*Hordeum vulgare* L.) by chlorophyll a fluorescence OLKJIP under drought stress and re-watering. Environ Exp Bot. 2007;60:438–446. https://doi.org/10.1016/j.envexpbot.2007.01.002
3. Xu L, Pan Y, Yu F. Effects of water stress on growth and physiological changes in *Pterocarya stenoptera* seedlings. Sci Hortic. 2015;190:11-23. https://doi.org/10.1016/j.scienta.2015.03.041
4. Kalaji HM, Račková L, Paganová V, Swoczyna T, Rusinkowski S, Sitko K. Can chlorophyll-a fluorescence parameters be used as bio-indicators to distinguish between drought and salinity stress in *Tilia cordata* Mill? Environ Exp Bot. 2018;152:149-157. https://doi.org/10.1016/j.envexpbot.2017.11.001
5. Dąbrowski P, Baczewska-Dąbrowska AH, Kalaji HM, Goltsev V, Paunov M, Rapacz M, et al. Exploration of chlorophyll a fluorescence and plant gas exchange parameters as indicators of drought tolerance in Perennial Ryegrass. Sensors 2019;19:2736. https://doi.org/10.3390/s19122736
6. Zhao J, Lang Y, Zhang S, Zhao Q, Zhang C, Xia J. Photosynthetic characteristics and chlorophyll a fluorescence transient in *Lonicera japonica* under drought stress. Acta Physiol Plant. 2019;41:124. https://doi.org/10.1007/s11738-019-2912-z
7. Vitale L, Francesca S, Arena C, D'Agostino N, Principio L, Vitale E, et al. Multitratis evaluation of a *Solanum pennellii* introgression tomato line challenged by combined abiotic stress. Plant biology 2023;25:518-528. https://doi.org/10.1111/plb.13518
8. Cetner MD, Pietkiewicz S, Podlaski S, Wiśniewski G, Chołuj D, Łukasik I, et al. Photosynthetic efficiency of Virginia mallow (*Sida Hermaphrodita* (L.) Rusby) under differentiated soil moisture conditions. Int J Sust Water Environ Syst. 2014;6:89-95. https://doi.org/10.5383/swes.06.02.010

9. Furtak K. Characteristics of Bacillus bacteria. In: Microbiological preparations for agricultural plants. Puławy, Poland: 2023 https://doi.org/10.26114/por.iung.2023.12.01https://www.iung.pl/publikacje/2023/preparaty_mikrobiologiczne_dla_roslin_rolniczych.pdf [in polish]
10. Consentino BB, Aprile S, Rouphael Y, Ntatsi G, De Pasquale C, Iapichino G, Alibrandi P, Sabatino L. Application of PGPB combined with variable N doses affects growth, yield-related traits, N-fertilizer efficiency and nutritional status of lettuce grown under controlled condition. Agronomy 2022;12:236. https://doi.org/10.3390/agronomy12020236
11. Dobrzyński J, Jakubowska Z, Kulkova I, Kowalczyk P, Kramkowski K. Biocontrol of fungal phytopathogens by *Bacillus pumilus.* Front Microbiol. 2023;14:1194606. https://doi.org/10.3389/ fmicb.2023.1194606
12. Dobrzyński J., Kulkova I., Jakubowska Z., Wróbel B. Non-native PGPB consortium altered the rhizobacterial community and slightly stimulated the growth of winter oilseed rape (*Brassica napus* L.) under field conditions. Microbial Ecology 2024;87:168. https://doi.org/10.1007/s00248-024-02471-3.
13. Guo J, Chi J. Effect of Cd-tolerant plant growth-promoting rhizobium on plant growth and Cd uptake by *Lolium multiflorum* Lam. and *Glycine max* (L.) Merr. in Cd-contaminated soil. Plant Soil 2014;375:205-214. https://doi.org/10.1007/s11104-013-1952-1
14. Pramanik K, Mirta S, Sarkar A, Maiti TK. Alleviation of phytotoxic effects of cadmium on rice seedlings by cadmium resistant PGPR strain Enterobacter aerogenes MCC 3092. J Hazard Mater. 2018;351:317-329. https://doi.org/10.1016/j.jhazmat.2018.03.009
15. Jouzani GS, Vilijanian E, Sharafı R. Bacillus thuringiensis: a successful insecticide with new environmental features and tidings. Appl Microbiol Biotechnol. 2017;101:2691-2711. https://doi.org/10.1007/s00253-017-8175-y
16. Strasser RJ, Srivastava A, Tsimili-Michael M. The influence transient as a tool to characterize and screenphotosynthetic samples, probing photosynthesis. In: Mechanism, Regulation and Adaptation. Edited by Yunus M, Pathre U, Mohanty P. Taylor and Francis Publishers, London, 2000; 443-480.
17. Dąbrowski P, Baczewska AH, Pawluśkiewicz B, Paunov, M.; Alexantrov, V.; Goltsev, V, et al. Prompt chlorophyll a fluorescence as a rapid tool for diagnostic changes in PSII structure inhibited by salt stress in Perennial ryegrass. J Photochem Photobiol B Biol. 2016;157:22–31. https://doi.org/10.1016/j.jphotobiol.2016.02.001
18. Meng L-L, Song J-F, Wen J, Zhang J, Wei J-H. Effects of drought stress on fluorescence characteristics of photosystem II in leaves of *Plectranthus scutellarioides*. Photosynh. 2016;53:414-421. https://doi.org/10.1007/s11099-016-0191-0
19. Schansker G, Strasser RJ. Quantification of non-QB-reducing centers in leaves using a far-red preillumination. Photosynth Res. 2005;84:145–151. https://link.springer.com/article/10.1007/s11120-004-7156-z
20. Baker NR, Oxborough K. Chlorophyll a Fluorescence a Signature of Photosynthesis. In: Chlorophyll fluorescence as a probe of photosynthetic productivity, edited by George Papaqeorgiou and Govindjee: Springer Publishers; 2004:66-79.
21. Olsen DJ, Shafiei R, Botha FC. The fast fluorescence kinetics: a sensitive tool for early detection of water stress in sugarcane. Proc Aust Soc Sugar Cane Technol. 2016;38:218-228. https://elibrary.sugarresearch.com.au/server/api/core/bitstreams/8441b297-1b30-4547-887b-fcf5af466bcd/content
22. Kalaji HM, Schansker G, Ladle RJ, Goltsev V, Bosa K, Allakhverdiev SI, et al. Frequently asked questions about in vivo chlorophyll fluorescence: practical issues. Photosynth Res. 2014;122:121-158. https://doi.org/10.1007/s11120-014-0024-6
23. Pollastrini M, Holland V, Brüggemann W, Bruelheide H, Dânilâ I, Jaroszewicz B, et al. Taxonomic and ecological relevance of the chlorophyll a fluorescence signature of tree species in mixed European forests. New Phytol. 2016;212:51–65. https://doi.org/10.1111/nph.14026
24. Baker NR. Chlorophyll fluorescence: a probe of photosynthesis in vivo. – Annu Rev Plant Biol. 2008;59:89-113. https://doi.org/10.1146/annurev.arplant.59.032607.092759
25. Dąbrowski P, Pawluśkiewicz B, Baczewska AH, Kalaji HM, Oglęcki P. Chlorophyll a fluorescence of perennial ryegrass (*Lolium perenne* L.) varieties under long term exposure to shade. Zemdirbyste-Agriculture 2015;102:305–312.
26. Guidi L, Calatayud A. Non-invasive tools to estimate stress-induced changes in photosynthetic performance in plants inhabiting Mediterranean areas. Environ Exp Bot. 2014;103:42–52. https://doi.org/10.1016/j.envexpbot.2013.12.007
27. Jankowski K, Sosnowski J, Jankowska J. Effect of hydrogel and different types of fertilizers on the number of turf shoots in lawns created by monocultures of red fescue (*Festuca rubra* L.) cultivars and its mixtures. Acta Agrobot. 2011;64:109-118.
28. Moreno-Galván AE, Cortés-Patiño S, Romero-Perdomo F, Uribe-Vélez D, Bashan Y, Bonilla RR. Proline accumulation and glutathione reductase activity induced by drought-tolerant rhizobacteria as potential mechanism to alleviate drought stress in Guinea grass. Appl Soil Ecol. 2020;147:103367. https://doi.org/10.1016/j.apsoil.2019.103367
29. Smagin AV, Sadovnikova NB, Belyaeva EA, Krivtsova VN, Shoba SA, Smagina MV. Gel-forming soil conditioners of combined action: field trials in agriculture and urban landscaping. Polymers 2022;14;5131 https://doi.org/10.3390/polym14235131

30. Truba M, Sosnowski J, Wróbel B, Krasnodębski P. Variability of soil microorganism numbers in response to exogenous organic matter and water-absorbing substrate. J Ecol Eng. 2024;25:223-233. https://doi.org/10.12911/22998993/181172
31. Bigelov CA, Macke GA, Johnson K, Richmond DS. Cool-season lawn performance as influenced by „Microclover" inclusion and supplemental nitrogen. ITSRJ 2020;14:121-132. https://doi.org/10.1002/its2.19
32. Saeedipooya E, Tehranifar A, Gazanchian A, Kazemi F, Shoor M. Evaluation of the replacement ability and comparison the growth and quality characteristics of clover lawn with common grass lawn in landscape to reduce the mowing costs. J Hortic Sci. 2022;53:15-28. https://doi.org/10.22067/jhs.2021.60852.0
33. IUSS Working Group WRB. 2022. World Reference Base for Soil Resources. International soil classification system for naming soils and creating legends for soil maps. 4th edition. International Union of Soil Sciences (IUSS), Vienna, Austria.
34. Patil C, Suryawanshi R, Koli S, Patil S. Improved method for effective screening of ACC (1-aminocyclopropane-1-carboxylate) deaminase producing microorganisms. J Microbiol Methods 2016;131:102-104. https://doi.org/10.1016/j.mimet.2016.10.009
35. Dworkin M, Foster J. Experiments with some microorganisms which utilize ethane and hydrogen. J Bacteriol. 1958;75:592-603. https://journals.asm.org/doi/pdf/10.1128/jb.75.5.592-603.1958
36. Penrose DM, Glick BR. Methods for isolating and characterizing ACC-deaminase containing plant growth-promoting rhizobacteria. Physiol Plantarum. 2003;118:10–15. https://doi.org/10.1034/j.1399-3054.2003.00086.x
37. Kalaji HM, Wołejko E, Łoboda T, Pietkiewicz S, Wyszyński Z. Chlorophyll fluorescence – new tool for photosynthetic performance evaluation of barley plants grown under different nitrogen rates. Zesz Probl Postęp Nauk Rol. 2004;496:375-383. [in Polish]
38. Digrado A, Bachy A, Mozaffar A, Schoon N, Bussotti F, Amelynck C, et al. Long-term measurements of chlorophyll a fluorescence using the JIP-test show that combined abiotic stresses influence the photosynthetic performance of the perennial ryegrass (*Lolium perenne*) in a managed temperature grassland. Physiol. Plant. 2017;161:355-371. https://doi.org/10.1111/ppl.12594
39. Michałek W, Kocira A, Findura P, Szparaga A, Kocira S. The influence of biostimulant Asahi SL on the photosynthetic activity of selected cultivars of *Phaseolus vulgaris* L. ROS 2018;20:1286-1310.
40. Szmagara M, Pudelska K, Durlak W, Marcinek B, Rojek K. The influence of Bio-algeen S90 on the growth of multiflora rose seedlings (*Rosa multiflora* Thumb.). Acta Sci Pol Hortorum Cultus 2019;18:35-43. https://doi.org/10.24326/asphc.2019.3.4
41. Murkowski A. Effect of some stress factors on chlorophyll luminescence in the photosynthetic apparatus crop plants. Monografia 61, Instytut Agrofizyki im. Bohdana Dobrzańskiego, Lublin, Poland: PAN Publishers; 2002. [in Polish] https://agro.icm.edu.pl/agro/element/bwmeta1.element.agro-article-d0408d83-970c-4217-9adc-35961b8e4040
42. Kalaji MH, Łoboda T. Chlorophyll fluorescence in plant physiological condition studies. Warszawa, Poland: SGGW Publishers; 2009. [in Polish]
43. Wang FG, Shi L, Zhang RY, Xu WZ, Bo YJ. Effect of nitrogen addition and *Bothriochloa ischaemum* and *Lespedeza davurica* mixture on plant chlorophyll fluorescence and community production in semi-arid grassland. Front Plant Sci. 2024;15:1400309. https://doi.org/10.3389/fpls.2024.1400309
44. Angelini G, Ragni P, Esposito D, Giardi P, Pompili ML, Moscardelli R. et al. A device to study the effect of space radiation on photosynthetic organisms. Phys Med. 2001;17:267-268.
45. Aazami MA, Asghari-Aruq M, Hassanpouraghdam MB, Ercisli S, Baron M, Sochor J. Low temperature stress mediates the antioxidants pool and chlorophyll fluorescence in *Vitis vinifera* L. cultivars. Plants 2021;10:1877. https://doi.org/10.3390/plants10091877
46. Li J, Bai X, Ran F, Zhang C, Yan Y, Li P, et al. Effects of combined extreme cold and drought stress on growth, photosynthesis, and physiological characteristics of cool-season grasses. Sci Rep. 2024;14:116. https://doi.org/10.1038/s41598-023-49531-
47. Schansker G, Tóth SZ, Holzwarth AR, Garab G. Chlorophyll a fluorescence: beyond the limits of the Q A-model. Photosynth Res. 2014;120:43–58. https://doi.org/10.1007/s11120-013-9806-5
48. Khan N, Essemine J, Hamdani S, Qu M, Lyu M-JA, Pereveen S, et al. Natural variation in the fast phase of chlorophyll a fluorescence induction curve (OJIP) in a global rice minicore panel. Photosynth Res. 2021;150:137-158. https://doi.org/10.1007/s11120-020-00794-z
49. Monneveux P, Pastenes C, Reynolds MP. Limitations to photosynthesis under light and heat stress in three high-yielding wheat genotypes. J Plant Physiol. 2003;160:657-666. https://doi.org/10.1078/0176-1617-00772
50. Mehta P, Jajoo A, Mathur S, Bharti S. Chlorophyll a fluorescence study revealing effects of high salt stress on Photosystem II in wheat leaves. Plant Physiol Biochem. 2010;48:16-20. https://doi.org/10.1016/j.plaphy.2009.10.006
51. Tsimilli-Michael M, Strasser R. The energy flux theory 35 years later: formulations and applications. Photosynth Res. 2013;117:289–320. https://doi.org/10.1007/s11120-013-9895-1
52. Sharma S, Bhatt U, Sharma J, Darkalt A, Mojski J, Soni V. Effect of different waterlogging periods on biochemistry, growth, and chlorophyll a fluorescence of *Arachis hypogaea* L. Front Plant Sci. 2022;13. https://doi.org/10.3389/fpls.2022.1006258

53. Jia H, Hao JB, Cao HB, Han Y, Li D, Chen HJ. Effects of shading on fast chlorophyll fuorescence induction dynamics of 'Baojiahong' peach leaves. Acta Bot Boreal -Occid Sin. 2015;35:1861–1867. https://doi.org/10.7606/j.issn.1000-4025.2015.09.1861
54. Chen SG, Yin CY, Dai XB, Sheng Q, Xu XM. Action of tenuazonic acid, a natural phytotoxin, on photosystem II of spinach. Environ Exp Bot. 2008;62:279–289. https://doi.org/10.1016/j.envex pbot.2007.10.002
55. Zhang DY, Pan XL, Mu GJ, Wang JL. Toxic effects of antimony on photosystem II of Synechocystis sp. as probed by in vivo chlorophyll fluorescence. J Appl Phycol. 2010;22:479–488. https://doi. org/10.1007/s10811-009-9482-1
56. Contreras-Porcia L, Thomas D, Flores V, Correa JA.Tolerance to oxidative stress induced by desiccation in *Porphyra columbina* (Bangiales, Rhodophyta). J Exp Bot. 2011;62:1815-1829. https://doi. org/10.1093/jxb/erq364

Figures

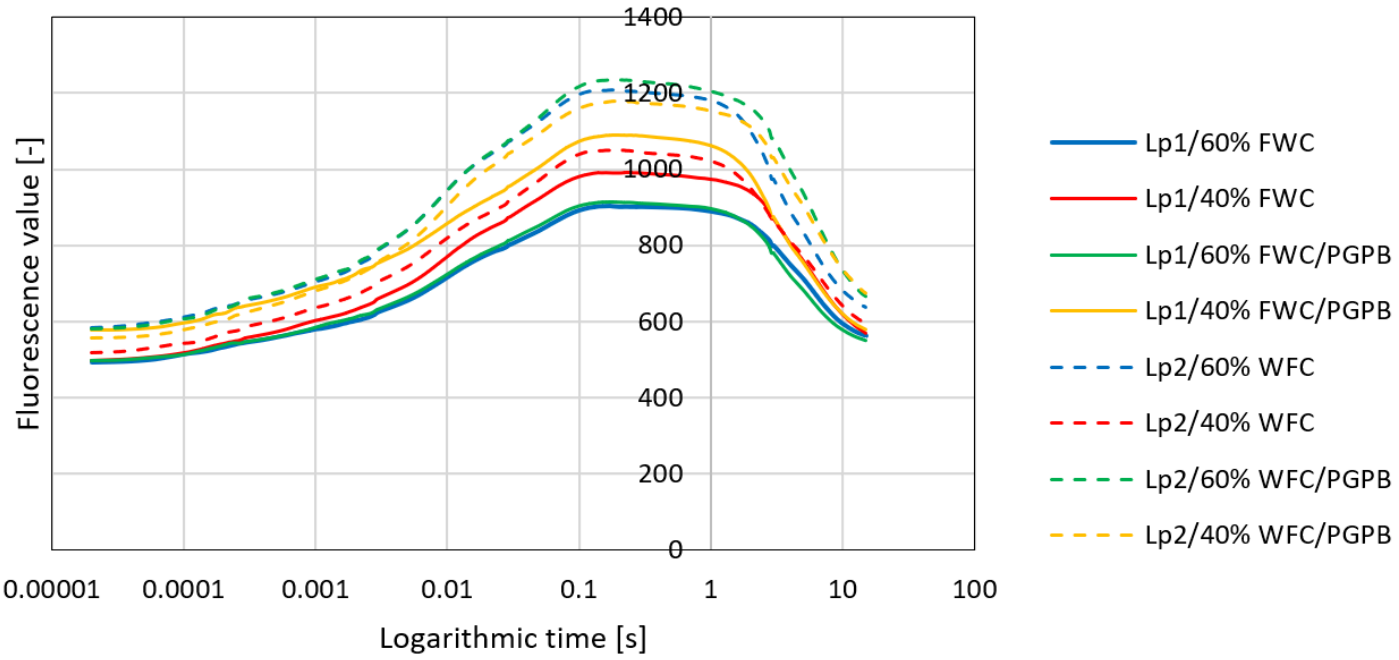

Figure 1

The effect of experimental factors on chlorophyll a fluorescence induction curve. Abbreviations: Lp1 – *Lolium perenne*in pure stand; Lp2 - *Lolium perenne*in a mixture with microclover; 40% FWC – 40% field water capacity; 60% FWC – 60% field water capacity; PGPB – Plant Growth Promoting Bacteria

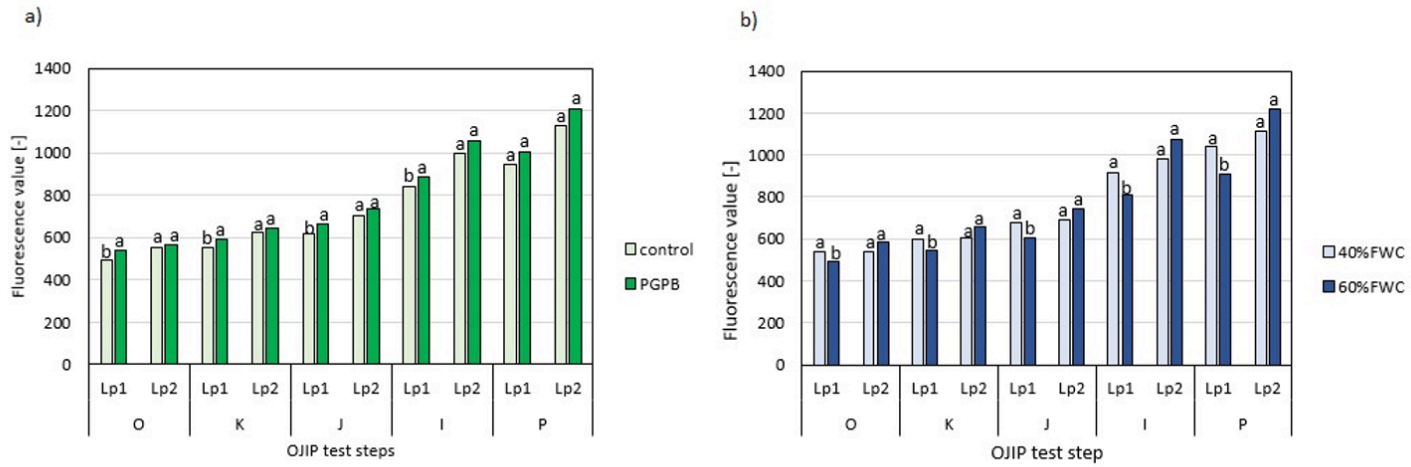

Figure 2

Effect of a) PGPB on chlorophyll a fluorescence assessed by the OJIP test; b) substrate moisture on chlorophyll fluorescence assessed by the OJIP test. Abbreviations: O ,K, J, I, P – points on the chlorophyll a fluorescence induction curve; Lp1 - *Lolium perene* grown in pure stand; Lp2 – *Lolium perene* grown in a mixture with microclover

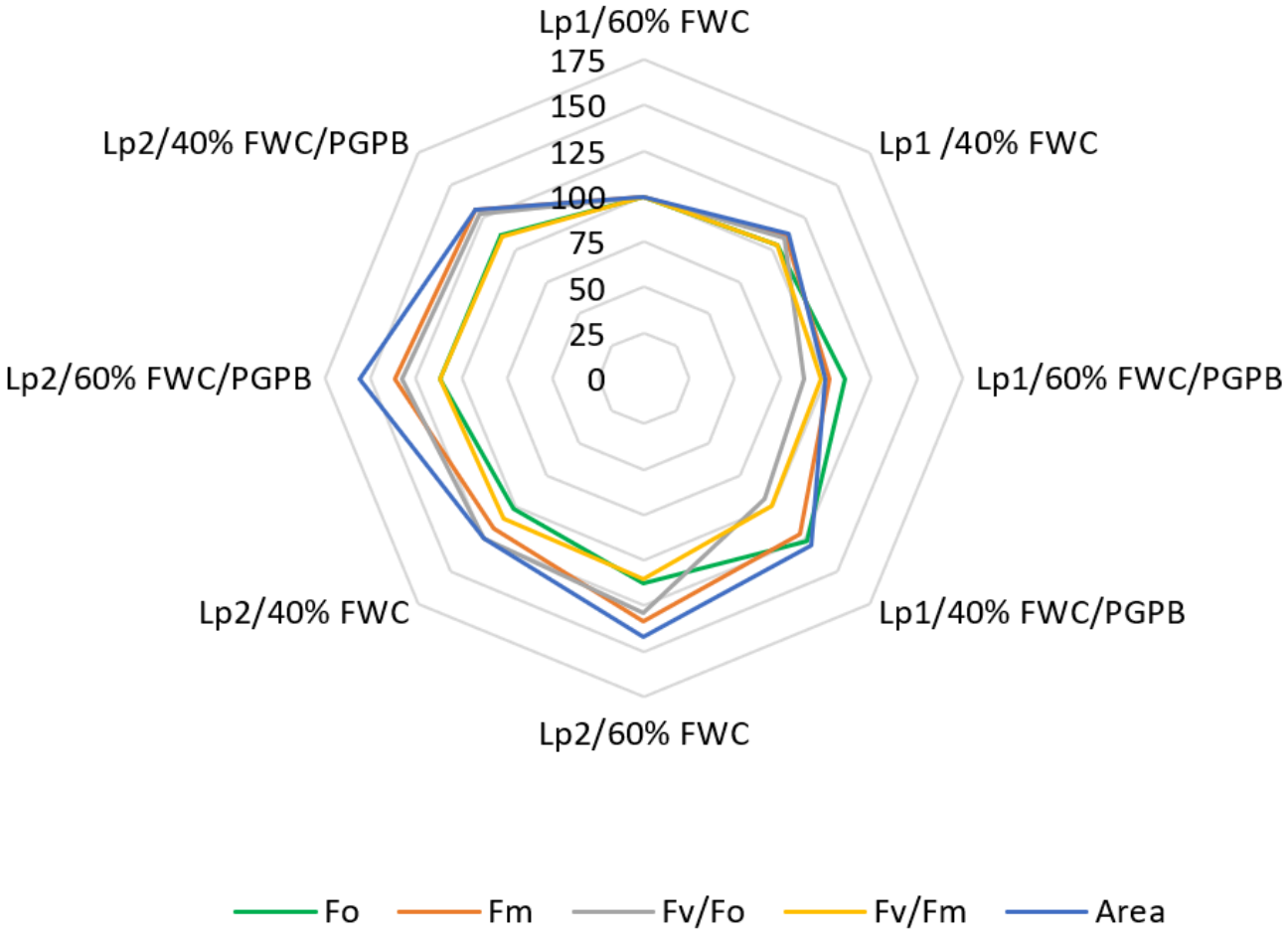

Figure 3

The effect of experimental factors on of Fo, Fm, Fv/Fo, Fv/Fm and Area parameters. Abbreviations: Lp1 – *Lolium perenne*in pure stand; LP2 - *Lolium perenne*in a mixture with microclover; 40% FWC – 40% field water capacity; 60% FWC – 60% field water capacity; PGPB – Plant Growth Promoting Bacteria

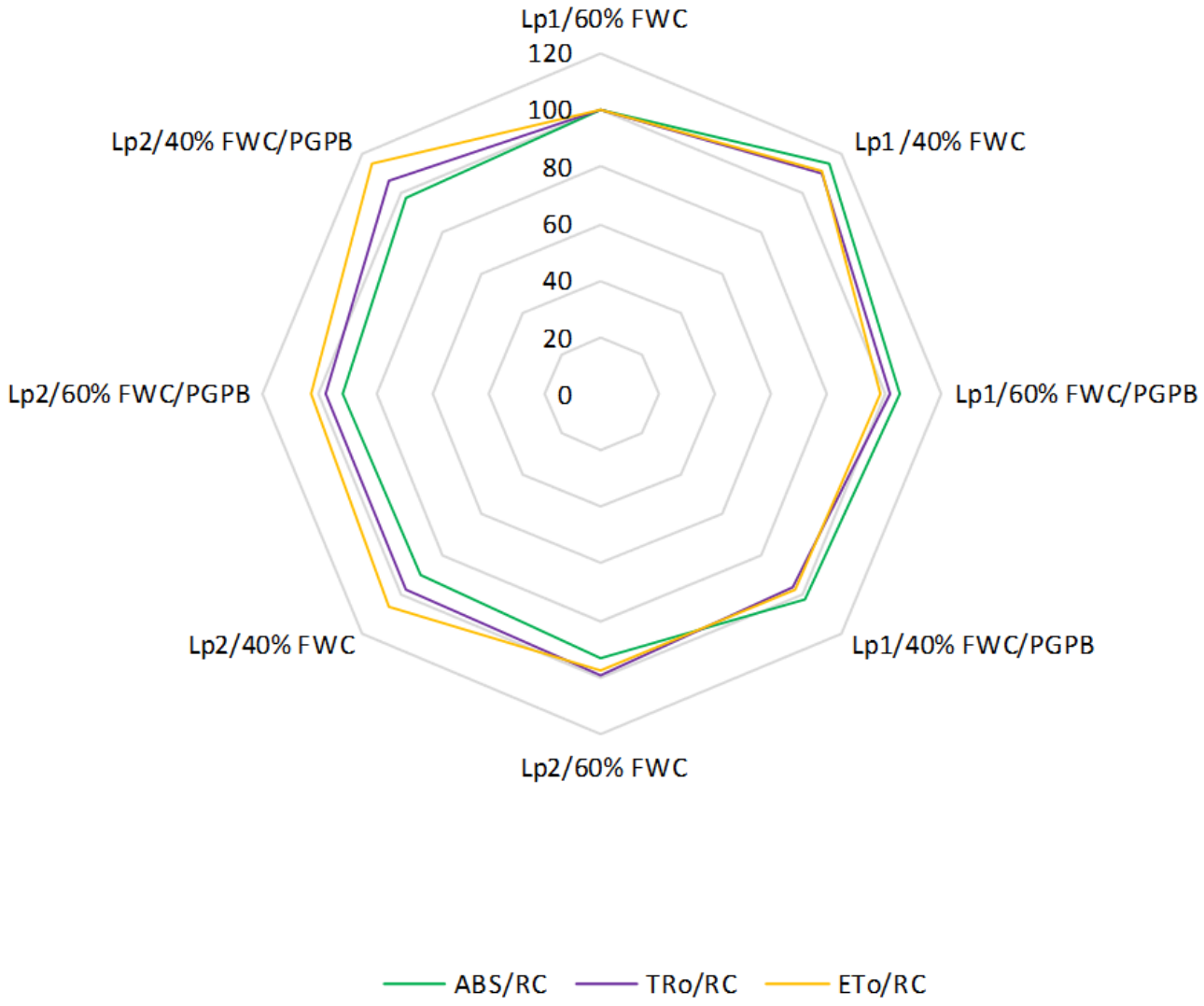

Figure 4

The effect of experimental factors on ABS/RC, TRo/RC, and ETo/RC parameters. Abbreviations: Lp1 – *Lolium perenne*in pure stand; LP2 - *Lolium perenne*in a mixture with microclover; 40% FWC – 40% field water capacity; 60% FWC – 60% field water capacity; PGPB – Plant Growth Promoting Bacteria

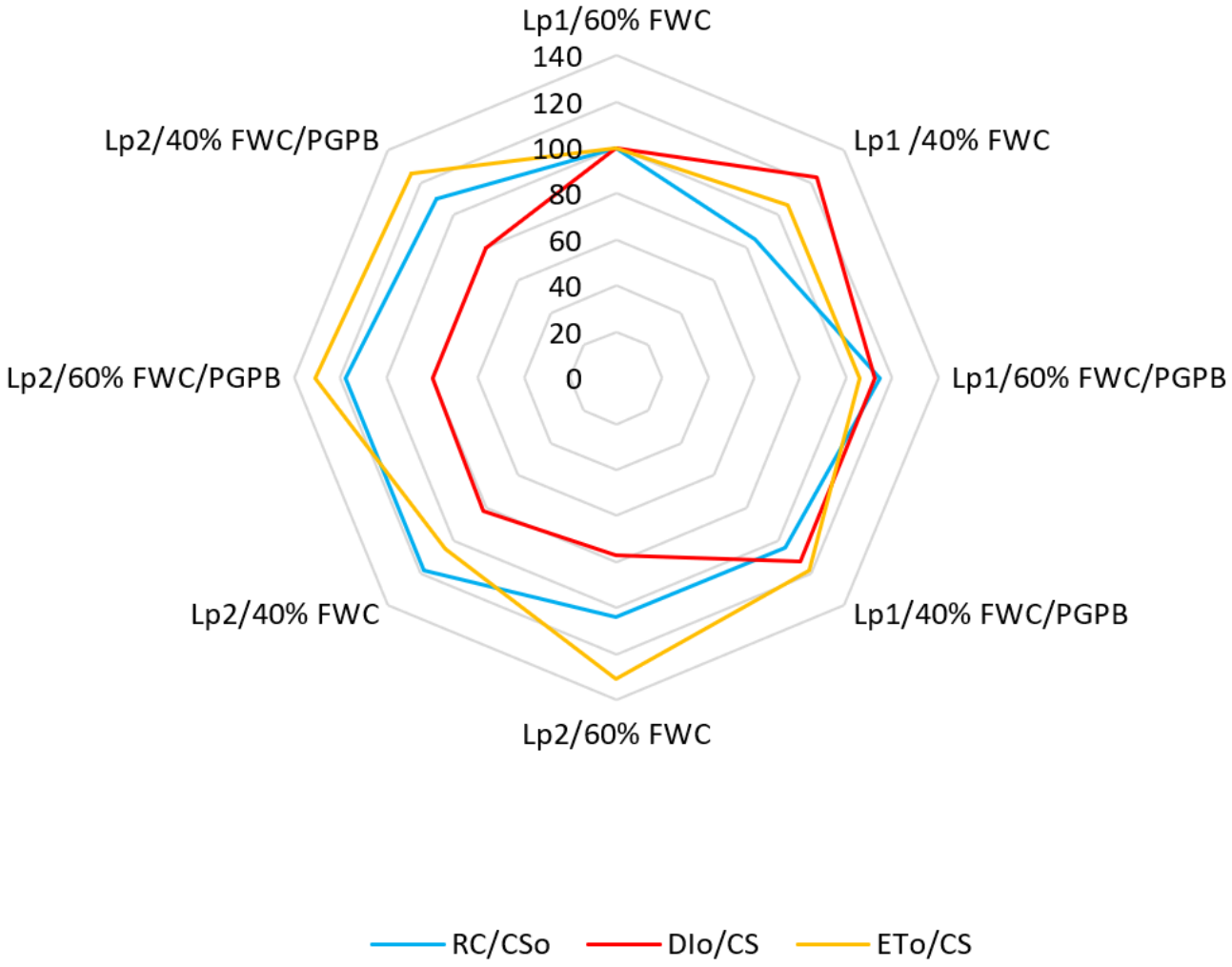

Figure 5

Effects of experimental factors on of RC/CSo, ETo/CSo, and DIo/CS parameters. Abbreviations: Lp1 – *Lolium perenne* in pure stand; LP2 - *Lolium perenne* in a mixture with microclover; 40% FWC – 40% field water capacity; 60% FWC – 60% field water capacity; PGPB – Plant Growth Promoting Bacteria