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Effects of plant growth-promoting bacteria on soil dehydrogenase activity and turfgrass aesthetics during early growth

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Highlights

- The addition of microclover to the mixture improved the visual quality of the lawn.
- Dehydrogenase activity was higher in optimally hydrated soil than in drought-stressed soil.
- Dehydrogenase activity was higher in soil from pots containing microclover.
- The use of PGPB had a positive effect on root length and dry mass.
- The use of PGPB increased the visual quality parameters of the lawn.

Abstract: This study evaluated the effects of a microbial inoculant containing plant growth-promoting bacteria (PGPB) on soil microbial properties and turfgrass aesthetic quality under limited irrigation. The experiment was conducted on two turfgrass systems: a perennial ryegrass (*Lolium perenne* L.) (T1) and a mixture including microclover (T2), under two soil moisture levels: 40 and 60% field water capacity (*FWC*).

The parameters analysed included regrowth intensity, root length-to-dry-mass ratio, soil-plant analysis development (SPAD) leaf greenness index, and soil dehydrogenase activity (DHA). PGPB application significantly increased both root system length and biomass, particularly under drought stress, which was also reflected in higher DHA values and improved turf visual quality. The highest dehydrogenase activity was recorded in PGPB-treated plots at 60% *FWC*, regardless of turf type. Although SPAD index differences were not statistically significant, a consistent trend indicated that PGPB application under drought conditions helped stabilise SPAD values.

The best visual quality was observed in the perennial ryegrass and microclover mixture, likely due to a more developed root system and enhanced microbial activity in the rhizosphere. These findings suggest that PGPB application at the beginning of the growing season can enhance turf resilience and recovery under drought stress.

Keywords: dehydrogenase activity, microclover, perennial ryegrass, plant growth-promoting bacteria, SPAD

INTRODUCTION

In recent years, growing attention has been directed toward the appearance and health of turfgrass lawns in both urban and rural settings. Turfgrass areas serve multiple functions, from sports fields to extensive shaded zones, and can also provide habitats that support biodiversity. As a result, researchers are seeking management strategies to maintain turf in good condition, protect vegetation from disease while preserving rhizosphere microbial activity and biodiversity within the sward. One such

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strategy involves reducing the use of mineral fertilisers in favour of biostimulant products. According to the EBIC (2012), biostimulants enhance nutrient use efficiency in plants by improving nutrient availability and supporting the production of essential compounds in the roots. This, in turn, enhances plant tolerance to abiotic stress and resistance to pests and diseases, while improving soil fertility, health, and structure. Among the various types of biostimulants, plant growth-promoting bacteria (PGPB) remain one of the least studied in the context of turfgrass management.

According to Maksimov *et al.* (2015), plant growth-promoting bacteria (PGPB) can be classified into three subgroups: (i) free-living bacteria that interact with plants under favourable conditions; (ii) bacteria inhabiting the rhizosphere (soil zones adjacent to roots) and the phyllosphere; and (iii) bacteria forming stable associations with certain plant tissues or organs, referred to as endophytic bacteria. As noted by Benedetto di *et al.* (2017), the use of PGPB offers environmental benefits by reducing the nutrient demands of plants. Soil inoculation with PGPB can improve plant growth directly – by facilitating the uptake of macro- and micronutrients and modulating plant hormone levels – or indirectly, by mitigating the inhibitory effects of various pathogens (Jakubowska, Gradowski and Dobrzyński, 2025; Naziębło and Dobrzyński, 2025). PGPB comprise diverse functional and taxonomic groups, including *Pseudomonas*, *Bacillus*, *Paenibacillus*, and *Rhizobium* (Stajković *et al*., 2011; Dobrzyński *et al*., 2024b; Dobrzyński *et al*., 2025; Dobrzyński and Kulkova, 2025). A key characteristic of these bacteria is their ability to mobilise nutrients bound in mineral or organic forms in the pedosphere and to fix atmospheric N_2, making it available to plants (Khabbaz *et al*., 2019; Dobrzyński *et al*., 2024a).

An essential factor in achieving a healthy and aesthetically pleasing lawn is seed-mixture composition. In temperate climates, perennial ryegrass (*Lolium perenne* L.) is commonly used as a base lawn species because it rapidly forms ground cover and regenerates readily. Breeding continues to improve desirable traits in this species, resulting in new turf cultivars (Christians, 2004; Hulke *et al*., 2007). When maintained under well-watered and fertile soil conditions, perennial ryegrass shows good persistence and disease resistance. As a result, 33,000 Mg of its seed are sold annually for lawn use in Europe. It is also used in other regions, particularly in North America, Australia, and New Zealand. However, diverse environmental conditions and/or usage types require different genetic backgrounds than those typically favoured in Europe (Sampoux *et al*., 2013). A notable advancement in recent years has been the introduction of white clover (*Trifolium repens sylvaticum* L.) in a dwarf form with small leaf blades, commonly referred to as microclover. Lawns incorporating microclover develop a dense, distinct structure and require less fertilisation than those composed solely of grasses (Wagner *et al*., 2010).

Soil water availability is a key determinant of plant growth and development. Water deficit reduces plant growth rates. Prolonged water deficit disrupts the plant's metabolism. According to the literature (Staniak, 2013), in studies on drought-resistance breeding in grasses, drought stress was imposed at 40% *FWC*. Additionally, Olszewska (2004) differentiated drought stress into different *FWC* levels depending on the soil. On mineral soil, drought stress was tested at 35% *FWC*, and on organic soil, drought stress was set at 40% *FWC*. However, in laboratory research methodology, it is much more common to find drought stress induced at 40% *FWC* on various types of soil (Kocoń, 2006; Olszewska, 2006; Olszewska, 2009; Staniak and Fariaszewska, 2014).

This study was initiated due to a lack of research on Bacillus-based PGPB in turf lawns. Specifically, no existing literature addresses the impact of PGPB on grass–microclover mixtures or the soil microbiological environment. Furthermore, this study seeks to explore plant-PGPB interactions under water-limited conditions, thereby contributing novel insights to the field.

MATERIALS AND METHODS

EXPERIMENTAL CONDITIONS

The experiment was carried out in pots from November 2023 to February 2024 in a growth chamber under controlled conditions (Photo 1). During the light period, the air temperature was maintained at 24 ±2°C, and during the dark period, at 16 ±2°C. Soil moisture was regulated at either 40 or 60% of field water capacity. These levels were selected according to the properties of the soil material used in the experiment. Light was provided by high-pressure sodium lamps at an intensity of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, with a 16 h photoperiod.

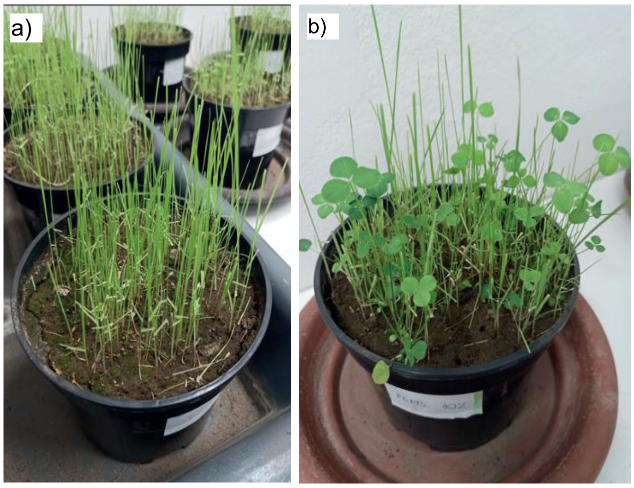

Photo 1. Two types of experimental plots: a) perennial ryegrass (T1), b) perennial ryegrass mixed with microclover (T2) (phot.: *M. Truba*)

Each pot, with a volume of 1.5 dm^3, was filled with soil material characterised by the following macronutrient concentrations: phosphorus – 0.70 $g \cdot kg^{-1}$ dry matter (DM), potassium – 1.05 $g \cdot kg^{-1}$ DM, and magnesium – 1.20 $g \cdot kg^{-1}$ DM. Micronutrient contents were as follows: iron – 4,550.00 $mg \cdot kg^{-1}$ DM, copper – 5.20 $mg \cdot kg^{-1}$ DM, manganese – 145.50 $mg \cdot kg^{-1}$ DM, and zinc – 14.10 $mg \cdot kg^{-1}$ DM. Phosphorus content was measured by the colorimetric method, while the other macro- and micronutrients were determined using the AAS method. Total nitrogen, analysed via the Kjeldahl method, equalled 1.31 $g \cdot kg^{-1}$ DM. Organic carbon (Corg) content, measured by the oxidation–titration method, was 12.00 $g \cdot kg^{-1}$ DM. The soil pH, determined in a 0.01 M $CaCl_2$ solution, was 6.40. Based on the Bouyoucos–Casagrande aerometric method, modified by Prószyński, the soil

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was classified as light clay (IUSS Working Group WRB, 2022). No additional fertilisation was applied during the course of the experiment.

Two turfgrass treatments were established. In the first (T1), perennial ryegrass (*Lolium perenne*) cv. 'Bokser' (FN Granum, Wodzierady, Poland) was sown in monoculture at a seeding rate of 36 g·m^{-2}. In the second treatment (T2), perennial ryegrass was sown at a slightly reduced rate of 34.9 g·m^{-2} and mixed with microclover (*Trifolium repens* cv. 'Pipolina') at 1.1 g·m^{-2}, resulting in a 3% microclover share in the total seed mixture.

EXPERIMENTAL TREATMENTS

The experimental units were subjected to the following treatments: at the onset of perennial ryegrass germination, a plant growth-promoting bacterial (PGPB) preparation was applied directly to the soil using a pipette. Once the plants reached the three-leaf growth stage, drought stress was imposed by maintaining soil moisture at 40% of field water capacity (*FWC*).

Based on these treatment combinations, the following experimental variants were established:

- T1/40%FWC – perennial ryegrass grown under drought stress at 40% *FWC*;
- T1/60%FWC – perennial ryegrass grown under optimal soil moisture at 60% *FWC*;
- T1/40%/PGPB – perennial ryegrass grown under drought stress at 40% *FWC* with PGPB application;
- T1/60%/PGPB – perennial ryegrass grown under optimal soil moisture at 60% *FWC* with PGPB application;
- T2/40%FWC – perennial ryegrass grown in mixture with microclover under drought stress at 40% *FWC*;
- T2/60%FWC – perennial ryegrass grown in mixture with microclover under optimal soil moisture at 60% *FWC*;
- T2/40%/PGPB – perennial ryegrass in mixture with microclover grown under drought stress at 40% *FWC* with PGPB application;
- T2/60%/PGPB – perennial ryegrass in mixture with microclover grown under optimal soil moisture at 60% *FWC* with PGPB application.

Each treatment was replicated four times, resulting in a total of 32 pot-grown experimental units.

The PGPB strain used in the study was *Bacillus* sp. N188 – an ACC deaminase-producing isolate obtained from soil at the Institute of Technology and Life Sciences – National Research Institute in Falenty (Poland). The 16S rRNA gene sequence of *Bacillus* sp. N188 has been deposited in GenBank under the accession number PP500625.

MEASUREMENTS AND OBSERVATIONS

Visual traits of the turfgrass – namely overall appearance, turf density, and regrowth intensity – were evaluated three times throughout the experiment, and the average values are presented in the results. Assessments were conducted using a 9-point scoring system for turfgrass (Prończuk, 1993), with the following rating scale:

- Overall appearance:
  1 – poor (no plants);
  3 – weak;
  5 – fair (low visual appeal);
  7 – good;
  9 – very good.
- Turf density (ground cover), with the authors' modification:
  1 – poor 0% cover;
  2 – 1–10% cover;
  3 – weak, large gaps between plants, 11–20% cover;
  4 – 21–50% cover;
  5 – fair 51–60% cover;
  6 – 61–70% cover;
  7 – good, small bare patches, 71–80% cover;
  8 – 81–90% cover;
  9 – very good, ideal carpet, 91–100% cover.
- Regrowth intensity:
  1 – no plants;
  2 – very intense (>300% of standard);
  3 – high (200–300% of standard);
  5 – moderate (100–200% of standard);
  7 – low (25–100% of standard);
  9 – very slow (<25% of standard).

All plots were mowed at the same time. Mowing was performed when grass in the 60% *FWC* reference treatment reached a height of 10 cm. The plants on each plot were cut at a height of 3 cm. Differences in plant height between the reference plot (T1 60% *FWC*) and plants in the other treatments were defined as regrowth intensity.

The leaf greenness index was measured once, at the end of the experiment. Flag leaf blades of perennial ryegrass were selected for soil-plant analysis development (SPAD) analysis using a Chlorophyll Meter SPAD-502 (Konica Minolta, Wrocław, Poland).

Root morphometric measurements covered the entire root system extracted from each pot. Roots were thoroughly washed to remove adhering soil and then separated by depth into three zones: 0–5 cm, 5–10 cm, and >10 cm. Fresh weight was recorded for each depth zone, followed by drying and weighing to determine root dry mass.

At the end of the experiment, dehydrogenase activity (DHA) was assessed. DHA was measured in soil samples collected from each pot, following the PN-ISO 23753-1 standard. The colorimetric method used 1% TTC (2,3,5-triphenyltetrazolium chloride) as a substrate, with samples incubated at 30°C for 24 h. Absorbance was measured at 485 nm, and results were expressed as μmol TPF·kg^{-1}·24 h^{-1} dry soil (Thalmann, 1968).

STATISTICAL ANALYSIS

Statistical analysis was performed using Statistica 13 software (TIBCO Software Inc., Palo Alto, CA, USA). Data were analysed at a significance level of $p < 0.05$. Differences between means were assessed using ANOVA, followed by Tukey's HSD test (honest significant difference). Statistically significant differences among means are indicated by different letters in the tables.

RESULTS AND DISCUSSION

Analysis of the overall appearance (Tab. 1) indicated that the highest-rated treatment was T2 treated with plant growth-promoting bacteria (PGPB) (Fig. 1b). Plants grown under optimal soil moisture conditions (60% *FWC*) received appearance ratings

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Table 1. General appearance depending on plant type, microbial inoculant application, and substrate moisture

Type of cultivated plant	Preparation	Humidity	
		40% *FWC*	60% *FWC*
T1	control	6.00 ±1.15 Ab	6.75 ±0.50 Aa
	PGPB	6.00 ±0.00 Ab	7.50 ±0.58 Aa
T2	control	4.75 ±0.50 Ab	8.50 ±0.58 Aa
	PGPB	6.50 ±1.29 Aa	8.25 ±0.50 Aa
Mean for preparation			
Control		5.37 Ab	7.62 Aa
PGPB		6.26 Ab	7.87 Aa
Mean for type of turf			
T1		6.00 Aa	7.12 Ba
T2		5.62 Ab	8.37 Aa

Explanations: T1 = perennial ryegrass; T2 = perennial ryegrass mixed with microclover; *FWC* = field water capacity; PGPB = plant growth-promoting bacteria; means in rows marked with different lowercase letters differ significantly at $p < 0.05$; means in columns marked with different uppercase letters differ significantly at $p < 0.05$.
Source: own study.

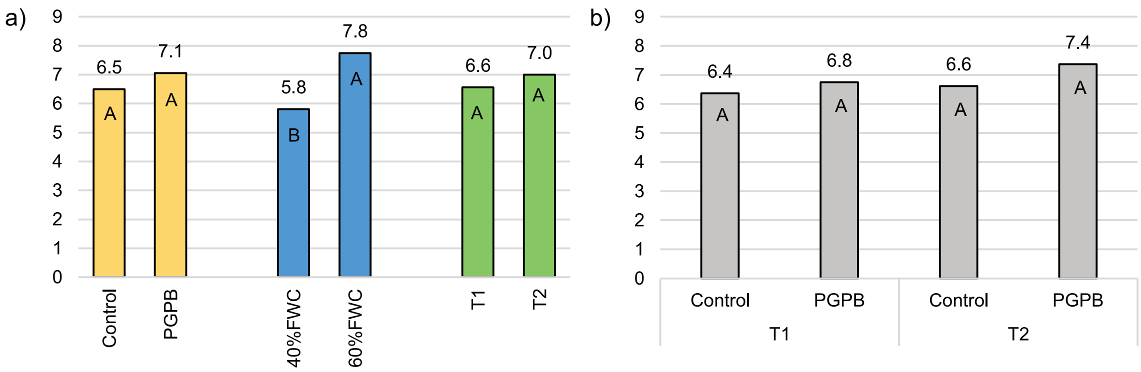

Fig. 1. General appearance of cultivated plants: a) single interaction effect for type of cultivated plants, substrate moisture level, and PGPB application; b) double interaction between type of cultivated plants and PGPB application; means within columns of the same colour marked with different uppercase letters differ significantly ($p < 0.05$); control = untreated control object; other symbols as in Tab. 1; source: own study

that were, on average, 25% higher than those grown under drought stress (40% *FWC*) (Fig. 1a). The use of PGPB improved the overall appearance score by approximately 14% during the drought stress period. Although the differences in mean overall appearance between PGPB-treated and untreated variants were not statistically significant, PGPB-treated plants scored 8% higher. A minor distinction was also observed between the two types of cultivated plants – on average, T2 was rated 6% higher than T1. The visual quality of turf is strongly influenced by adequate NPK fertilisation. In this experiment, NPK fertilisation was deliberately omitted to better isolate the effects of microclover inclusion and PGPB application. According to Bigelow *et al.* (2022), a grass–clover mixture can produce sustainable and visually acceptable turf while reducing the need for mineral nitrogen fertilisation. Supporting this, Oberson *et al.* (2013) suggest that microclover has the ability to fix dinitrogen (N_2), which is subsequently released into the soil through the reduction process. The well-documented symbiotic relationship between legume roots and certain rhizobacteria, which results in nodule formation and nitrogen fixation, plays an important role in this process (Matse *et al.*, 2020). In this context, PGPB bacteria may have enhanced nitrogen availability to plants. Rhizobia and PGPB often coexist in the rhizosphere and interact during root colonisation (Benedetto di *et al.*, 2017). Positive effects of the interaction between rhizobia and PGPB were also reported by Saharan and Nehra (2011), who found that PGPB may facilitate nodule formation and enhance nitrogen fixation in leguminous roots.

The evaluation of turf coverage (Tab. 2) revealed that the highest scores were achieved by the T2 plant type (perennial ryegrass mixed with microclover) treated with the PGPB microbial inoculant (Fig. 2b). Plants grown at 60% *FWC* exhibited, on average, an 18% higher turf coverage score (Fig. 2a). The use of PGPB improved the turf cover of cultivated plants in experimental plots by approximately 8% during drought stress. Likewise, T2 treatments containing microclover achieved, on average, a 14% higher rating. As noted by Bigelow *et al.* (2022), turfgrass requires a steady nitrogen supply to sustain green colour and seasonal shoot density. In this context, the presence of microclover in the mixture likely contributed additional nitrogen to the turfgrass. Moreover, the application of PGPB further

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Table 2. Turf cover depending on the type of plant cultivated, microbial inoculant application and substrate moisture

Type of cultivated plant	Preparation	Humidity	
		40% *FWC*	**60% *FWC***
Mean			
T1	control	6.00 ±0.00 Aa	7.00 ±0.00 Aa
	PGPB	6.50 ±0.58 Aa	7.50 ±0.58 Aa
T2	control	6.75 ±0.50 Ab	8.50 ±0.58 Aa
	PGPB	7.25 ±1.26 Aa	8.25 ±0.50 Aa
Mean for preparation			
Control		6.37 Ab	7.75 Aa
PGPB		6.87 Aa	7.87 Aa
Mean for type of turf			
T1		6.25 Ab	7.25 Ba
T2		7.00 Ab	8.37 Aa

Explanations as in Tab. 1.
Source: own study.

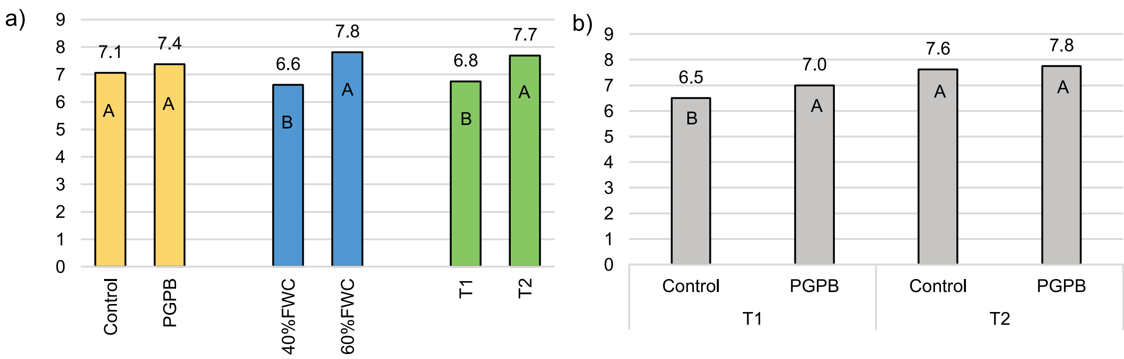

Fig. 2. Turf cover: a) single interaction for type of cultivated plants, substrate moisture, and PGPB application; b) two-way interaction between type of cultivated plants and PGPB application; means in columns of the same colour marked with different uppercase letters differ significantly at $p < 0.05$; other symbols as in Tab. 1; source: own study

improved the uptake of macro- and micronutrients, supporting overall plant development and tillering. Microclover has become increasingly popular for its ability to increase turfgrass density; its growth habit effectively fills gaps between grass plants, improving visual uniformity (Wagner *et al.*, 2010).

Analysis of regrowth intensity (Tab. 3) revealed that the slowest growth rate was observed in T1 plots, whereas T2 plots supplemented with PGPB showed markedly more intensive regrowth (>30%) (Fig. 3b). Plants grown in pots with 60% *FWC* received 29% lower scores due to their faster growth compared to those grown at 40% *FWC* (Fig. 3a). In addition, supplementation with the PGPB microbial strain resulted in an average increase of 20% in growth intensity compared to control treatments. The obtained results indicate that both the application of PGPB and maintaining optimal substrate moisture contributed to increased regrowth intensity. The use of PGPB increased the regrowth intensity of cultivated plants by approximately 17.5% during drought stress. In the case of lawn grass varieties, slow regrowth is desirable. The intensification of regrowth by PGPB is an interesting finding, suggesting that the application of these bacteria may accelerate the renewal and regeneration of plants under drought stress. Previous studies have shown that PGPB positively affects the growth rate of various plant species (Benedetto di *et al.*, 2017; Ding *et al.*, 2024), including grasses (Rahnama *et al.*, 2023). However, in the context of turfgrass, a lower regrowth intensity is actually a desirable trait due to the reduced need for mowing. It is worth emphasising that the introduction of microclover in the present experiment did not increase regrowth intensity. The microclover remained low-growing and did not contribute to an increased mowing frequency.

Analysis of root dry weight and length (Fig. 4) under optimal substrate moisture conditions showed that the most developed root systems were found in the plots containing microclover and supplemented with PGPB. Under reduced irrigation, the most extensive root systems were also observed in plots with microclover. For the grass-only plots, there was a tendency for plants treated with PGPB to develop shorter but heavier root systems under drought stress conditions. According to Li *et al.* (2022), PGPB inoculation may alleviate drought stress by modifying root morphology and activating SOD (superoxide dismutase) activity. Pooya *et al.* (2022) also confirmed that most grass mixtures with microclover had more extensive root systems and greater drought resistance than commercial turfgrass systems.

Relative chlorophyll content on the experimental treatments was assessed based on the soil-plant analysis development (SPAD)

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Table 3. Regrowth intensity depending on the application of microbial inoculant and substrate moisture

Type of cultivated plant	Preparation	Humidity	
		40% *FWC*	60% *FWC*
T1	control	9.00 ±0.00 Aa	5.00 ±0.00 Ab
	PGPB	8.75 ±0.50 Aa	5.00 ±0.50 Ab
T2	control	8.25 ±0.96 Aa	5.50 ±0.58 Ab
	PGPB	7.75 ±0.96 Aa	4.50 ±0.58 Ab
Mean for preparation			
Control		8.62 Aa	6.37 Ab
PGPB		7.12 Aa	4.87 Ab
Mean for type of turf			
T1		8.87 Aa	5.12 Ab
T2		6.87 Ba	6.12 Aa

Explanations as in Tab. 1.
Source: own study.

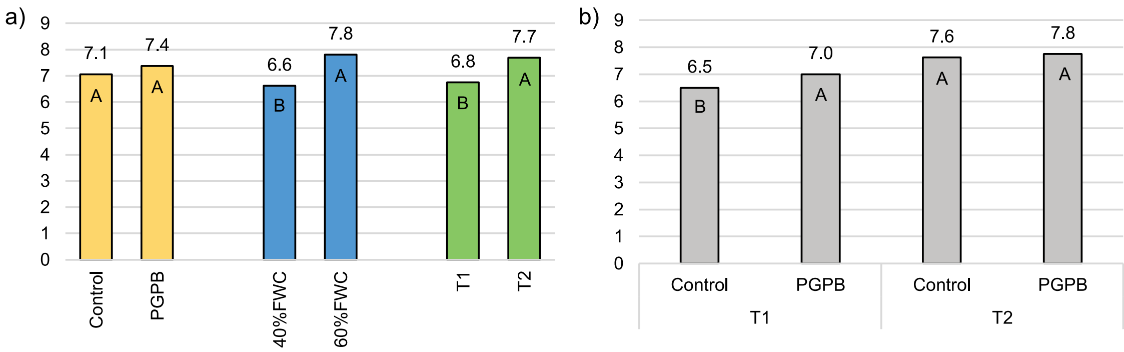

Fig. 3. Regrowth intensity: a) single interaction for type of cultivated plants, substrate moisture, and PGPB; b) double interaction between type of cultivated plants and PGPB; means in columns of the same colour marked with different uppercase letters differ significantly at $p < 0.05$; other symbols as in Tab. 1; source: own study

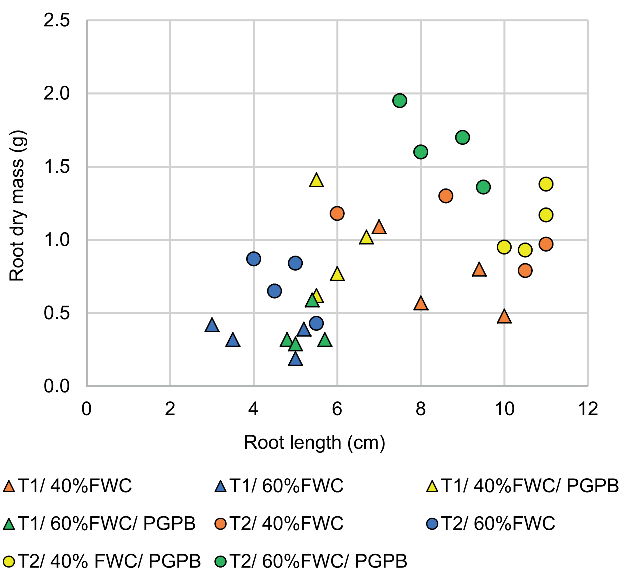

Fig. 4. Ratio of root length to root dry mass; symbols as in Tab. 1; source: own study

leaf greenness index. Statistical analysis of the SPAD parameter (Fig. 5) showed no significant differences in the multifactor interaction between turf type, treatment, and substrate moisture, nor in the other single and double interactions. SPAD values ranged from 31.9 to 41.4 (Tab. 4). The highest SPAD values were recorded in plants grown in soil with 40% *FWC* without PGPB support. However, the data suggest a trend in which PGPB application under drought stress brought SPAD values closer to those observed under optimal moisture conditions. This indicates that the plants in these plots had larger cells and looser leaf tissue structures, which caused a reduction in compound concentrations, including chlorophyll. This effect is a result of plant growth under favourable environmental conditions.

According to current literature, plants subjected to drought stress may exhibit higher SPAD values because their tissues shrink due to water deficiency, leading to denser tissue structures, which translates into a higher SPAD index (Kacperska, 1998; Olszewska *et al.*, 2010; Staniak and Baca, 2018). In contrast, in well-watered plants, the tissues are properly hydrated and loosened, which results in lower SPAD values. The literature indicates that a high chlorophyll content in the leaves of grasses growing under water stress conditions is a plant's defence mechanism against drought.

Measurement of microbial enzymatic activity in the experimental treatments (Tab. 5) showed that significant changes occurred only under 40% *FWC*. The multifactor interaction between soil moisture, microbial inoculant application, and type of cultivated plants demonstrated that, at 40% *FWC*, the

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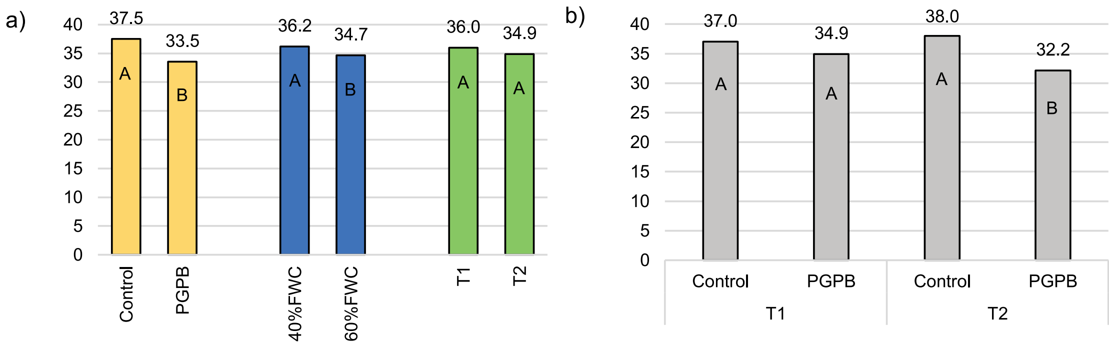

Fig. 5. Soil-plant analysis development (SPAD) leaf greenness index: a) single interaction for type of cultivated plants, substrate moisture, and PGPB; b) double interaction between perennial ryegrass and PGPB; means in columns of the same colour marked with different uppercase letters differ significantly at $p < 0.05$; symbols as in Tab. 1; source: own study

Table 4. Soil-plant analysis development (SPAD) leaf greenness index depending on the application of microbial inoculant and substrate moisture

Type of cultivated plant	Humidity	Preparation	
		40% *FWC*	60% *FWC*
T1	control	38.37 ±3.85 Aa	35.70 ±3.18 Aa
	PGPB	32.65 ±3.01 Aa	37.30 ±1.59 Aa
T2	control	41.42 ±14.78 Aa	33.47 ±8.29 Aa
	PGPB	32.37 ±12.57 Aa	31.92 ±6.69 Aa
Mean for preparation			
Control		39.90 Aa	34.74 Aa
PGPB		32.47 Aa	34.61 Aa
Mean for type of turf			
T1		35.47 A	36.50 A
T2		36.90 A	32.59 A

Explanations as in Tab. 1.
Source: own study.

application of PGPB significantly increased dehydrogenase activity (DHA) in both T1 and T2 soils by about 36% compared to the control. No statistically significant differences were found in the main effects of plant type, soil moisture level, or microbial treatment (Fig. 6a). The highest DHA values in both T1 and T2 plots were observed following PGPB application (Fig. 6b).

Higher DHA values were generally observed in plots with 60% *FWC*. Soil temperature and moisture are the most important

Table 5. Dehydrogenase enzymatic activity (μmol TPF·kg^{-1}·24 h^{-1} dry soil) depending on the application of microbial inoculant and substrate moisture

Type of cultivated plant	Preparation	Humidity	
		40% *FWC*	**60% *FWC***
T1	control	0.248 Ba	0.521 Aa
	PGPB	0.677 Aa	0.718 Aa
T2	control	0.206 Ba	0.441 Aa
	PGPB	0.553 Aa	0.621 Aa
Mean for preparation			
Control		0.400 Aa	0.571 Aa
PGPB		0.441 Aa	0.580 Aa
Mean for type of turf			
T1		0.379 Aa	0.531 Aa
T2		0.462 Aa	0.620 Aa

Explanations as in Tab. 1.
Source: own study.

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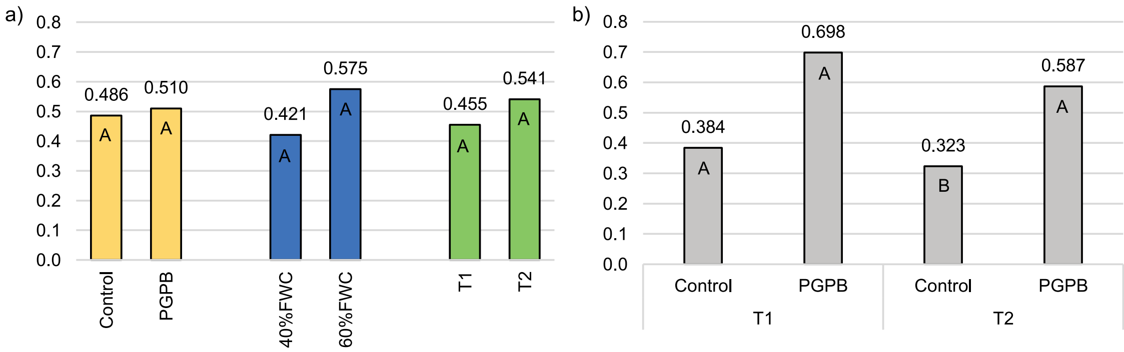

Fig. 6. Dehydrogenase enzymatic activity (μmol TPF·kg^{-1}·24 h^{-1} dry soil): a) single interaction for type of cultivated plants, substrate moisture, and PGPB; b) double interaction between type of cultivated plants and PGPB; means in columns of the same colour marked with different uppercase letters differ significantly at $p < 0.05$; symbols as in Tab. 1; source: own study

factors influencing microbial life and thus soil enzymatic activity (Nowak and Kąklewski, 2003). That study confirmed that the relative enzymatic activities closest to those in native soils were found in samples with 60% *FWC*.

DHA measurement in soil is an indicator of the intensity of microbial respiratory metabolism, primarily that of bacteria and actinomycetes. According to Gałązka and Kocoń (2015), the highest dehydrogenase activity was obtained after the application of microbial preparations such as EM and UGmax. The authors concluded that both the microbial preparation and study year both significantly increased soil dehydrogenase activity. However, the mostly non-significant differences in DHA values found in other studies may result from high soil fertility in the experimental setup. Swędrzyńska *et al.* (2015) also observed similar DHA values across all treatments without statistically significant differences. They concluded that the uniform results were due to high inherent soil fertility. According to Siwik-Ziomek and Koper (2006), full mineral and organic fertilisation with lime and magnesium contributed to creating optimal conditions for enhancing the biochemical potential of soil microflora. A high DHA value indicates favourable conditions in the soil environment that support microbial activity and development.

Although mineral and organic fertilisers were not applied in this experiment, the introduced PGPB may have contributed to releasing macro- and micronutrients previously bound in the soil, making them available and absorbable by the plants.

CONCLUSIONS

The cultivation of perennial ryegrass in combination with microclover received better visual ratings. This was likely due to a significantly more developed root system, both in mass and structure, as well as richer microbial life in the soil, which improved nutrient availability for the plants.

Maintaining substrate moisture at 60% *FWC* had a positive impact on plant growth. Optimal soil moisture improved visual parameters such as general appearance and plant density for perennial ryegrass (‘Bokser’ variety) and microclover (‘Pipolina’ variety). Higher moisture levels also favoured microbial development, as indicated by the significant increase in dehydrogenase enzymatic activity (DHA).

Drought stress caused deterioration in most of the characteristics studied in perennial ryegrass (‘Bokser’ variety) and microclover (‘Pipolina’ variety). Drought stress was alleviated in treatments where PGPB was applied. Overall appearance and turf cover improved, regrowth intensity increased, and soil dehydrogenase activity was higher.

The positive effect of PGPB on perennial ryegrass (‘Bokser’ variety) and microclover (‘Pipolina’ variety) plants in the early stages of growth under laboratory conditions adds new, previously unexplored information to the scientific literature. This opens up the possibility of further research into the use of PGPB in later stages of plant growth and under field conditions.

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CONFLICT OF INTERESTS

All authors declare that they have no conflict of interests.

REFERENCES

Benedetto di, N.A. *et al.* (2017) “The role of plant growth promoting bacteria in improving nitrogen use efficiency for sustainable crop production: a focus on wheat,” *AIMS Microbiology*, 3(3), pp. 413–434. Available at: https://doi.org/10.3934/microbiol.2017.3.413.

Bigelow, C.A. *et al.* (2022) “Cool-season lawn performance as influenced by ‘Microclover’ inclusion and supplemental nitrogen,” *International Turfgrass Society Research Journal*, 14(1), pp. 121–132. Available at: https://doi.org/10.1002/its2.19.

Christians, N.E. (2004) “Cool-season grasses,” in *Fundamentals of turfgrass management*. Wiley, pp. 33–58.

Ding, S. *et al.* (2024) “Less is more: A new strategy combining nanomaterials and PGPB to promote plant growth and phytoremediation in contaminated soil,” *Journal of Hazardous Materials*, 469, 134110. Available at: https://doi.org/10.1016/j.jhazmat.2024.134110.

Dobrzyński, J. *et al.* (2024a) “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*, 87(1), 168. Available at: https://doi.org/10.1007/s00248-024-02471-3.

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This is an open access article under the CC BY-NC-ND license (https://creativecommons.org/licenses/by-nc-nd/4.0/)

Dobrzyński, J. *et al.* (2024b) "*Pseudomonas* sp. G31 and *Azotobacte*r sp. PBC2 changed structure of bacterial community and modestly promoted growth of oilseed rape," *International Journal of Molecular Sciences*, 25(23), 13168. Available at: https://doi.org/10.3390/ijms252313168.

Dobrzyński, J. *et al.* (2025) "Non-native PGPB consortium consisting of *Pseudomonas* sp. G31 and *Azotobacter* sp. PBC2 promoted winter wheat growth and slightly altered the native bacterial community," *Scientific Reports*, 15(1), 3248. Available at: https://doi.org/10.1038/s41598-025-86820-3.

Dobrzyński, J. and Kulkova, I. (2025) "*Paenibacillus peoriae*: current knowledge and agricultural biotechnology potential of a close relative of *P. polymyxa*," *Antonie van Leeuwenhoek*, 118(9), 120. Available at: https://doi.org/10.1007/s10482-025-02135-3.

EBIC (2012) *What are biostimulants*? Brussels: European Biostimulants Industry Council. Available at: http://www.biostimulants.eu/about/what-are-biostimulants (Accessed: March 15, 2025).

Gałązka, A. and Kocoń, A. (2015) "Ocena efektywności działania preparatów z mikroorganizmami pożytecznymi na aktywność enzymatyczną gleby [Evaluation of the effectiveness of preparations with beneficial microorganisms on the enzymatic activity of the soil]," *Studia i Raporty IUNG-PIB*, 45(19), pp. 143–154. Available at: https://greentribepeople.com/wp-content/uploads/2025/01/Wplyw-EM-na-aktywnosc-enzymatyczna-gleby.pdf (Accessed: September 5, 2025).

Hulke, B.S. *et al.* (2007) "Winterhardiness and turf quality of accessions of perennial ryegrass (*Lolium perenne* L.) from public collections," *Crop Science*, 47(4), pp. 1596–1602. Available at: https://doi.org/10.2135/cropsci2006.10.0671.

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 edn. Vienna, Austria: International Union of Soil Sciences. Available at: https://files.isric.org/public/documents/WRB_fourth_edition_2022-12-18.pdf (Accessed: September 5, 2025).

Jakubowska, Z., Gradowski, M. and Dobrzyński, J. (2025) "Role of plant growth-promoting bacteria (PGPB) in enhancing phenolic compounds biosynthesis and its relevance to abiotic stress tolerance in plants: A review," *Antonie van Leeuwenhoek*, 118(9), 123. Available at: https://doi.org/10.1007/s10482-025-02130-8.

Kacperska, A. (1998) "Reakcje roślin na czynniki stresowe [Plant responses to stress]," in J. Kopcewicz and S. Lewak (eds.) *Postawy fizjologii roślin [Basics of plant physiology]*. Warszawa: PWN, pp. 575–633.

Khabbaz, S.E. *et al.* (2019) "Plant Growth Promoting Bacteria (PGPB) – A versatile tool for plant health management," *Canadian Journal of Pesticides & Pest Management*, 1(1), pp. 1–25. Available at: https://doi.org/10.34195/can.j.ppm.2019.05.001.

Kocoń, A. (2006) "Wpływ stresu wodnego na fotosyntezę wybranych odmian pszenicy jarej [The effect of water stress on photosynthesis of chosen spring wheat cultivars]," *Zeszyty Problemowe Postępów Nauk Rolniczych*, 509, pp. 133–139. Available at: https://bibliotekanauki.pl/articles/802137.pdf (Accessed: March 20, 2025).

Li, Y. *et al.* (2022) "Indigenous PGPB inoculant from Qinghai-Tibetan plateau soil confer drought-stress tolerance to local grass *Poa annua*," *International Journal of Environmental Research*, 16, 85. Available at: https://doi.org/10.1007/s41742-022-00470-1.

Maksimov, I.V. *et al.* (2015) "Plant growth-promoting bacteria in regulation of plant resistance to stress factors," *Russian Journal of Plant Physiology*, 62, pp. 715–726. Available at: https://doi.org/10.1134/S1021443715060114.

Matse, D.T. *et al.* (2020) "Effects of coinoculaiton of *Rhizobium* with plant growth promoting rhizobacteria on the nitrogen fixation and nutrient uptake of *Trifolium repens* in low phosphorus soil," *Journal of Plant Nutrition*, 43(5), pp. 739–752. Available at: https://doi.org/10.1080/01904167.2019.1702205.

Naziębło, A. and Dobrzyński, J. (2025) "Biotransformation of As, Cr, Hg, and Mn by *Pseudomonadota*: chances and risks," *Biodegradation*, 36(4), 60. Available at: https://doi.org/10.1007/s10532-025-10157-x.

Nowak, J. and Kąklewski, K. (2003) "Wpływ różnych warunków przechowywania gleby na zmiany aktywności wybranych enzymów [Influence of different soil storage conditions on changes in activity of selected enzymes]," *Zeszyty Problemowe Postępów Nauk Rolniczych*, 492, pp. 225–232. Available at: https://bibliotekanauki.pl/articles/808067 (Accessed: September 20, 2025).

Oberson, A. *et al.* (2013) "Nitrogen fixation and transfer in grass – clover leys under organic and conventional cropping system," *Plant and Soil*, 371, pp. 237–255. Available at: https://doi.org/10.1007/s11104-013-1666-4.

Olszewska, M. (2004) "Reakcja koniczyny białej uprawianej na dwóch typach gleb na stres wodny [Reaction of white clover grown on two types of soil to water stress]," *Acta Scientiarum Polonorum. Agricultura*, 3(2), pp. 203–213. Available at: https://yadda.icm.edu.pl/agro/element/bwmeta1.element.dl-catalog-757ac4af-3d47-45ab-8560-5b0da166f987 (Accessed: May 22, 2025).

Olszewska, M. (2006) "Effect of water stress on physiological processes, leaf greenness (SPAD index) and dry matter yield of *Lolium perenne* and *Dactylis glomerata*," *Polish Journal of Natural Sciences*, 21(2), pp. 533–562. Available at: https://www.cabidigitallibrary.org/doi/full/10.5555/20073147891 (Accessed: August 7, 2025).

Olszewska, M. (2009) "Reakcja odmian kostrzewy łąkowej (*Festuca pratensis* Huds.) i tymotki łąkowej (*Phleum pratense* L.) uprawianych na glebie organicznej na niedobór wody [Response of cultivars of meadow fescue (*Festuca pratensis* Huds.) and timothy (*Phleum pratense* L.) grown on organic soil to moisture deficiency]," *Acta Scientiarum Polonorum. Agricultura*, 8(1), pp. 37–46. Available at: https://bibliotekanauki.pl/articles/47355.pdf.

Olszewska, M. *et al.* (2010) "Porównanie reakcji wybranych gatunków traw na stres wodny [Comparison of the response of selected grass species to water stress]," *Łąkarstwo w Polsce*, 13, pp. 127–136. Available at: https://bibliotekanauki.pl/articles/76153.pdf (Accessed: April 5, 2025).

Pooya, E.S. *et al.* (2022) "Evaluation of drought resistance of grass and clover mictures to introduce cover plants with lower maintenance," *Journal of Plant Production Research*, 28(4), pp. 105–122.

Prończuk, S. (1993) "System oceny traw gazonowych [Evaluation system for turf grasses]," *Biuletyn Instytutu Hodowli i Aklimatyzacji Roślin*, 186, pp. 127–132.

Rahnama, S. *et al.* (2023) "Seed priming with plant growth-promoting bacteria (PGPB) improves growth and water stress tolerance of *Secale montanum*," *Heliyon*, 9(4), e15498. Available at: https://doi.org/10.1016/j.heliyon.2023.e15498.

Sampoux, J.P. *et al.* (2013) "Breeding perennial ryegrass (*Perennial ryegrass* L.) for turf usage: an assessment of genetic improvements in cultivars released in Europe, 1974–2004," *Grass and Forage Science*, 68(1), pp. 33–48. Available at: https://doi.org/10.1111/j.1365-2494.2012.00896.x.

Saharan, B.S. and Nehra, V. (2011) "Plant growth promoting rhizobacteria: A critical review," *Life Sciences and Medicine*

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Research, 2011, LSMR-21. Available at: https://www.researchgate.net/profile/Houda-Kawas/post/do_any_body_know_about_induced_systemic_Resistance_in_plants_by_Streptomyces_spp/attachment/59d641e479197b807799dc2b/AS%3A436719616040960%401481133478064/download/7791af3a7e8cb1cd165e22bd6b67b47c7aca.pdf (Accessed: August 26, 2025).

Siwik-Ziomek, A. and Koper, J. (2006) "Ksztaltowanie aktywnosci dehydrogenaz w glebie plowej po zmianie nawozenia [Forming of dehydrogenase activity in lessive soil after changing of fertilization]," *Zeszyty Problemowe Postępów Nauk Rolniczych*, 512, pp. 521–527. Available at: https://bibliotekanauki.pl/articles/800105.pdf (Accessed: August 26, 2025).

Stajković, O. *et al.* (2011) "Improvement of common bean growth by co-inoculation with *Rhizobium* and plant growth-promoting bacteria," *Romanian Biotechnological Letters*, 16(1), pp. 5919–5926. Available at: https://www.academia.edu/download/39495043/Improvement_of_common_bean_growth_by_co-20151028-26584-zcaqkx.pdf (Accessed: March 25, 2025).

Staniak, M. (2013) *Reakcja wybranych gatunków i odmian traw pastewnych na niedobór wody w glebie [Reaction of selected species and varieties of forage grasses to soil water deficiency]*. Puławy: Dział Upowszechniania i Wydawnictw IUNG-PIB.

Staniak, M. and Baca, E. (2018) "Plon suchej masy i białka oraz indeks zieloności liścia SPAD koniczyny łąkowej i festulolium w zależności od sposobu uprawy i poziomu wilgotności gleby [Dry matter and protein yield and SPAD leaf greenness index of red clover and festulolium depending on the cultivation method and soil moisture level]," *Polish Journal of Agronomy*, 34, pp. 34–43. Available at: https://doi.org/10.26114/pja.iung.359.2018.34.04.

Staniak, M. and Fariaszewska, A. (2014) "Produktywność traw pastewnych w warunkach suszy [Forage quality under drought conditions]," *Studia i Raprty IUNG-PIB*, 41(15), pp. 195–208. Available at: https://doi.org/10.26114/sir.iung.2014.41.13.

Swędrzyńska, D. *et al.* (2015) "Wpływ biokondycjonera glebowego Soleflor na żywotność i plonowanie kupkówki pospolitej (*Dactylis glomerata* L.) oraz stan środowiska mikrobiologicznego gleby [Influence of the Soleflor soil bioconditioner on the microbiological state of soil and the vigour and yield of Orchard Grass (*Dactylis glomerata* L.)]," *Annual Set The Environment Protection*, 17, pp. 1320–1338. Available at: https://bibliotekanauki.pl/articles/1818574.pdf (Accessed: April 9, 2025).

Thalmann, A. (1968) "Zur Methodik der Bestimmung der Dehydrogenaseaktivität im Boden mittels Triphenyltetrazoliumchlorid (TTC) [Methodology for determining dehydrogenase activity in soil using triphenyltetrazolium chloride (TTC)]," *Landwirtschaftliche Forschung*, 21, pp. 249–258.

Wagner, M. *et al.* (2010) "Microclover – use of small-leaved clover on lawns," *European Journal of Turfgrass Science*, 41(1), pp. 3–8. Available at: https://www.cabidigitallibrary.org/doi/full/10.5555/20103125954 (Accessed: March 1, 2025).

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