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ARTICLE

RESEARCH ARTICLE

Sunlight, LED and HPS lighting modulate *Bacillus mycoides* response to shade stress in perennial ryegrass

La luz solar y la iluminación LED y HPS modulan la respuesta de *Bacillus mycoides* al estrés por sombra en raigrás perenne

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Abstract: *Aim of study*: To investigate the combined effects of supplemental lighting technologies, light-emitting diode (LED) and high-pressure sodium (HPS), perennial ryegrass cultivars, and *Bacillus mycoides* inoculation on early turfgrass establishment under stadium-like shade conditions, addressing critical renovation timelines in professional sports venues. *Area of study*: Greenhouse facility at Ege University's Bayındır Vocational School, Izmir, Türkiye. *Material and methods*: Three perennial ryegrass cultivars *Lolium perenne* L.'Presidian', *Lolium perenne* L. 'Apple SGL', and *Lolium × boucheanum* Kunth. 'Solstice II', were grown in washed sand (0-2 mm) under four light environments: Sunlight, 70% Shade, Shade + LED, and Shade + HPS. *B. mycoides* strain BM02 was applied at 2 L ha^{-1} to half the treatments. Both supplemental lighting systems provided 100 µmol m^{-2} s^{-1} photosynthetic photon flux density. Parameters measured included canopy cover, growth rate, root development, Dark Green Colour Index (DGCI), and chlorophyll content over five weeks using a 4×3×2 factorial design with four replicates. *Main results*: Significant three-way interactions dominated most responses. Shade yielded poorest performance across all parameters. LED lighting achieved canopy cover approaching sunlight levels (43.5-51.2% vs 48.6-57.3%), while HPS resulted in significantly lower coverage (20.2-28.3%) despite promoting vertical growth. *B. mycoides* enhanced canopy cover (5.8-21.3%) and fresh root weight (23.4-28.5%) under supplemental lighting but reduced canopy cover (11.3-12.8%) and DGCI (11.3-24.2%) under sunlight conditions. *Research highlights*: LED lighting provides superior establishment outcomes with 40% energy savings compared to HPS. *B. mycoides* benefits are maximized under stress conditions rather than optimal environments.

Keywords: *Bacillus mycoides*; LED lighting; perennial ryegrass; PGPR; shade stress; sports turf; supplemental lighting.

Resumen: *Objetivo del estudio*: Investigar los efectos combinados de las tecnologías de iluminación suplementaria, diodo emisor de luz (LED) y sodio de alta presión (HPS), los cultivares de raigrás perenne, y la inoculación con *Bacillus mycoides* en el establecimiento temprano del césped bajo condiciones de sombra similares a las de un estadio, abordando los plazos críticos de renovación en recintos deportivos profesionales. *Área de estudio*: Instalaciones de invernadero en la Escuela Vocacional de Bayındır de la Universidad de Ege, Izmir, Turquía. *Material y métodos*: Tres cultivares de raigrás perenne, *Lolium perenne* L. 'Presidian', *Lolium perenne* L. 'Apple SGL' y *Lolium × boucheanum* Kunth. 'Solstice II', se cultivaron en arena lavada (0–2 mm) bajo cuatro ambientes lumínicos: luz

solar, 70% de sombra, sombra + LED y sombra + HPS. La cepa BM02 de *B. mycoides* se aplicó a una dosis de 2 L ha^{-1} en la mitad de los tratamientos. Ambos sistemas de iluminación suplementaria proporcionaron 100 µmol m^{-2} s^{-1} de densidad de flujo fotónico fotosintético. Los parámetros medidos incluyeron cobertura del tapiz, tasa de crecimiento, desarrollo radicular, índice de color verde oscuro (DGCI) y contenido de clorofila durante cinco semanas, utilizando un diseño factorial 4 × 3 × 2 con cuatro repeticiones. *Resultados principales*: Las interacciones significativas de tres factores dominaron todas las variables de respuesta. La sombra produjo el rendimiento más bajo en todos los parámetros. La iluminación LED alcanzó una cobertura del tapiz cercana a los niveles de luz solar (43.5–51.2% frente a 48.6–57.3%), mientras que HPS resultó en una cobertura significativamente menor (20.2–28.3%) a pesar de promover el crecimiento vertical. *Bacillus mycoides* incrementó la cobertura del tapiz (5.8–21.3%) y el peso fresco radicular (23.4–28.5%) bajo iluminación suplementaria, pero redujo la cobertura del tapiz (11.3–12.8%) y el índice de color verde oscuro (DGCI) (11.3–24.2%) bajo condiciones de luz solar. *Aspectos destacados de la investigación*: La iluminación LED proporciona resultados superiores en el establecimiento con un ahorro energético del 40% en comparación con HPS. Los beneficios de *B. mycoides* se maximizan bajo condiciones de estrés más que en ambientes óptimos.

Palabras clave: *Bacillus mycoides*; césped deportivo; estrés por sombra; iluminación LED; iluminación suplementaria; PGPR; raigrás perenne.

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Supplementary information ↓

1. INTRODUCTION

Light serves a dual role for plants, acting as both the primary energy source for photosynthesis and a critical environmental signal regulating growth, development and photomorphogenesis. The quantity and quality of light significantly influence plant growth rates, morphology, and physiological processes (Taiz et al., 2015). However, modern architectural designs, particularly in sports stadia, often limit direct sunlight exposure to playing surfaces, creating significant challenges for turfgrass management (Turnbull, 2021). Baker (1995) reported that shade was considered a major problem at 35% of football venues and a moderate problem at 48% of venues in the UK. Such shading impairs photosynthetic efficiency, leading to canopy thinning, reduced root biomass, and declines in wear tolerance, pest resistance and physiological vigor (Brito et al., 2023).

Turfgrasses adapt to shade by exhibiting characteristic physiological and morphological changes. These often include altered chlorophyll content (Xie et al., 2020), typically with a lower chlorophyll a/b ratio (Gardner & Goss, 2013), and modifications to leaf ultrastructure designed to maximize light capture under low irradiance (Wherley et al., 2005). While these adaptations represent attempts to optimize photosynthetic light harvesting and carbon gain, most turfgrass species remain poorly adapted to prolonged shade (Bell, 2011).

To mitigate these limitations, supplemental controlled lighting systems have been implemented to overcome these challenges. Although high-pressure sodium (HPS) lamps have traditionally been used, their high energy costs and uneven spectral output (Islam et al., 2012) have encouraged a shift toward tuneable light-emitting diode (LED) systems. LEDs not only enhance turf quality and wear tolerance

but also consume 30-40% less energy (Brito et al., 2023). However, the impact of different light environments, particularly during the critical germination and seedling establishment phases, remains relatively underexplored. This early phase is critical given the demanding renovation timelines often required in professional sports venues.

Lolium perenne L., despite its widespread use in stadiums due to rapid germination, is classified as highly shade-intolerant among cool-season turfgrasses (Gardner & Goss, 2013), exhibiting impaired photosynthetic capacity and reduced tillering under suboptimal light (Dąbrowski et al., 2015). Current research has focused mainly on mature turfgrass responses to light quality, overlooking germination and seedling establishment period. Brito et al. (2023) investigated how different LED light qualities (i.e., varying red, green, and blue ratios) and intensities influence photosynthetic rates, pigment concentrations, and energy efficiency in mature perennial ryegrass, seeking a balance between physiological performance and energy consumption for sports turf. Abélard & Galbrun (2022) found that LED and HPS lighting similarly enhanced turf quality, canopy cover, and wear tolerance in mature stands. However, the physiological adjustments and biomass allocation patterns during the critical early stages of seedling development (i.e., the first 3-4 weeks after germination) under different light conditions remain poorly understood. This gap in literature is particularly important for stadium turf management, where rapid and complete establishment is required within 20 days or less, highlighting the need for further research focused on this early period.

Plant growth-promoting rhizobacteria (PGPR) represent a complementary biological approach to enhance turfgrass stress tolerance by modulating host plant physiology under suboptimal light conditions. These microorganisms are applied to enhance nutrition efficiency, abiotic stress tolerance, and crop quality traits (Du Jardin, 2015). However, the exact ways in which different biostimulants affect plants are often complex and not fully understood. The results can vary depending on the specific interactions between the plant, the bacterium, and the environment. *Bacillus mycoides*, a PGPR, may improve turfgrass establishment under restricted light conditions. *B. mycoides* enhances root development and abiotic stress resilience through phytohormone modulation and improved nutrient solubility (Kurniawan & Chuang, 2022; Tsotetsi et al., 2022).

While supplemental lighting partially mitigates shade-induced physiological limitations, microbial augmentation could further improve photosynthetic performance, resource allocation, and stress resilience. However, the specific interactions between different lighting technologies, bacterial inoculation, and cultivar genetics during early establishment have not been investigated.

This study investigated the effects of four light environments (1) Sunlight, (2) 70% Shade, (3) shade supplemented with LED lighting (Shade + LED), and (4) shade supplemented with HPS lighting (Shade + HPS), along with *B. mycoides* inoculation on the early development of three commercial cultivars of perennial ryegrass, *Lolium perenne* L. 'Presidian' (P) and 'Apple SGL' (SGL), and *Lolium × boucheanum* Kunth. 'Solstice II' (S). Key establishment traits were measured, including growth rate, canopy cover, colour, root length, root biomass, and chlorophyll content (chlorophyll a, chlorophyll b, total chlorophyll, and the chlorophyll a/b ratio). Given the known sensitivity of perennial ryegrass to shade, it was hypothesized that supplemental lighting would improve early growth compared to shade by enhancing photosynthetic capacity and favourably altering growth morphology. In addition, *B. mycoides* was expected to promote better establishment, especially in terms of canopy cover, colour and root development, particularly under Shade + LED, Shade + HPS, and sunlight environments.

2. MATERIALS AND METHODS

2.1. Experimental setup

The experiment was conducted over five weeks in the greenhouse facility of Ege University's Bayındır Vocational School, Izmir, Türkiye. The greenhouse provided semi-controlled environmental conditions

with ambient temperatures maintained between 16-20°C. The experimental units consisted of 12 × 17 cm (diameter × height) plastic pots filled with washed sand with a particle size of 0-2 mm as the growth medium. Three commercial cultivars of perennial ryegrass, *Lolium perenne* L. 'Presidian' and 'Apple SGL' and *Lolium × boucheanum* Kunth. 'Solstice II', which are commonly used on football pitches, were used in the study. Seeds were sown at a rate of 50 g m^{-2}. The fertilizer A 22-5-11 NPK was applied once at the beginning of the experiment. *Bacillus mycoides* strain BM02 (1.1 × 10^9 CFU) was applied at a rate of 2 L ha^{-1} to inoculated treatments.

The experiment was carried out in a 4 × 3 × 2 factorial design with four replicates comprising 96 experimental units. Factors were: (1) Light environment: full sunlight (Sunlight), 70% shade using a neutral density cloth (Shade), Shade supplemented with LED lighting (Shade + LED), and Shade supplemented with high-pressure sodium lighting (Shade + HPS). (2) Cultivar: (P, SGL, and S), (3) Microbial inoculation (*Bacillus mycoides* vs. uninoculated control).

2.2. Light treatments

The full spectrum LED fixture (Model: ANKA, 340 Watt; Globalgrowlight, Istanbul, Türkiye) was positioned 1.5 meters above the top surface of the pots, as per manufacturer recommendations. The HPS lamp (600 Watt; Secret Jardin, Manage, Belgium) was positioned 2 meters above the surface of the pots, following the standard application used in football field practices. Both lighting fixtures were calibrated to provide approximately 100 µmol m^{-2} s^{-1} photosynthetic photon flux density at the canopy level, resulting in a measured Daily Light Integral of 6.48 mol·m^{-2}·d^{-1}. To prevent light contamination between treatments, LED, HPS, and Shade environments were physically separated using light-proof plastic mulch barriers. The sunlight treatment was positioned at a distance from the shade cloth installation to ensure complete exposure to ambient greenhouse lighting without interference from shading materials.

2.3. Data collection

Plants were trimmed to 3–4 cm when they reached 6–7 cm in height. Plant height measured before every mowing and the average daily cumulative vertical growth rate was calculated by dividing the total vertical growth over the experimental period by the number of days, accounting for regular mowing events. Digital photographs were taken in a lightbox (Nikon D3400, Focal length 18 mm, Camera settings were: centre autofocus, shutter speed: 1/50, aperture F-stop 11, ISO 800). Dark Green Colour Index (DGCI) values were determined using Turf Analyzer software (Karcher et al., 2017). Canopy cover was assessed using Canopeo software (Patrignani & Ochsner, 2015).

At the end of the experiment, the plants were removed from the pots, the substrate was cleansed, and the length of the roots were measured (cm). Roots were blotted dry and weighed using a precision scale (0.0000 g) to determine fresh root yield. Samples were then oven-dried at 105°C for 48 hours to determine dry root weight per pot.

Chlorophyll content was estimated according to the method described by Uddin et al. (2012). In summary, fresh leaf samples (200 mg) were cut into small pieces and submerged in 20 mL of 80% acetone, then stored in the dark for 72 hours. The absorbance of the resulting solution was measured spectrophotometrically at 645 nm and 663 nm. Chlorophyll content was calculated and expressed as mg g^{-1} fresh weight (FW) using the equations below (1, 2, 3):

$$\text{Chlorophyll a } \left(\text{mg g}^{-1}\text{FW}\right) = \frac{12.7\text{A}_{663} - 2.69\text{A}_{645}}{1000} \times \frac{\text{V}}{\text{W}} \quad (1)$$

$$\text{Chlorophyll b } \left(\text{mg g}^{-1}\text{FW}\right) = \frac{22.9\text{A}_{645} - 4.86\text{A}_{663}}{1000} \times \frac{\text{V}}{\text{W}} \quad (2)$$

$$\text{Total chlorophyll } \left(\text{mg g}^{-1}\text{FW}\right) = \frac{20.2\text{A}_{645} + 8.02\text{A}_{663}}{1000} \times \frac{\text{V}}{\text{W}} \quad (3)$$

Where:

A_{645} = Absorbance at 645 nm

A_{663} = Absorbance at 663 nm

V = Final volume of the acetone extract (mL)

W = Fresh weight of the leaf sample (g)

Data were analyzed using GraphPad Prism version 10.2.3 (GraphPad Software, Boston, Massachusetts USA, www.graphpad.com.). Three-way ANOVA was conducted for each dependent variable to evaluate the fixed effects of light environment (L), cultivar (C), and *B. mycoides* inoculation (I), including their interactions. Significant main effects or interactions ($p \leq 0.05$) were further explored using post-hoc pairwise comparisons with Sidak adjustments to control the family-wise error rate ($p \leq 0.05$).

3. RESULTS

Three-way ANOVA revealed that establishment responses were dominated by significant three-way interactions (L × C × I), indicating that none of these factors act independently during early turfgrass development (Table 1). Shade environment consistently yielded the lowest values for all parameters except chlorophyll a/b compared to other light conditions, while sunlight generally supported the highest levels of establishment parameters (Table 2). Among artificial light treatments, LED lighting typically resulted in better performance than HPS, although improvement varied depending on the parameter and cultivar.

Table 1. Three-way ANOVA results for effects of light environment (L), ryegrass cultivar (C), and *Bacillus mycoides* inoculation (I) on turfgrass establishment parameters

Factors	dF	Canopy Cover	DGCI	Growth Rate	Root Length	Fresh Root Weight	Dry Root Weight	Chl a	Chl b	Total Chl	Chl a/b
		F	*F*	*F*	*F*	*F*	*F*	*F*	*F*	*F*	*F*
L	3	8002.57***	325.71***	720.28***	3380.85***	7984.28***	1218.41***	113.72***	59.28***	106.79***	2.90*
C	2	142.89***	47.65***	24.46***	95.85***	197.78***	38.56***	111.35***	80.99***	121.09***	10.27***
I	1	267.28***	135.20***	13.65***	371.43***	144.75***	24.11***	0.64	7.07*	3.78	7.92**
LxC	6	5.86***	16.11***	3.97**	24.66***	12.51***	3.41**	3.16*	1.69	2.58*	1.48
LxI	3	33.59***	55.10***	7.92***	138.04***	19.51***	2.63	3.25*	0.65	1.73	0.85
CxI	2	8.91***	15.98***	1.39	0.52	0.14	0.09	0.46	0.97	0.09	3.80*
LxCxI	6	2.64*	8.86***	1.52	0.52	2.84*	2.36*	3.72**	5.07***	4.51***	5.68***

DGCI = Dark Green Colour Index, Chl: Chlorophyll , *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$.

Table 2. Effects of light environment (L), ryegrass cultivar (C), and *Bacillus mycoides* inoculation (I) on turfgrass establishment parameters (Mean ± SE, n = 4). Ryegrass cultivars, P: 'Presidian', SGL: 'Apple SGL', S: 'Solstice II'

Treatments	Canopy Cover	DGCI	Fresh Root Weight	Dry Root Weight	Chl a	Chl b	Total Chl	Chl a/b
Unit	%		g	g	mg g^{-1}	mg g^{-1}	mg g^{-1}	
Shade+LED								
P								
Control	46.8±0.31	0.49±0.01	2.39±0.03	0.28±0.01	1.78±0.06	1.12±0.03	2.91±0.09	1.59±0.02
B. mycoides	49.5±0.54	0.50±0.01	2.95±0.06	0.38±0.01	1.88±0.07	1.21±0.03	3.08±0.05	1.56±0.09
SGL								
Control	50.8±0.29	0.56±0.01	2.59±0.07	0.31±0.01	1.52±0.04	0.92±0.04	2.44±0.08	1.66±0.03
B. mycoides	51.2±0.45	0.47±0.01	3.24±0.09	0.41±0.01	1.65±0.01	1.14±0.06	2.79±0.07	1.46±0.08
S								
Control	43.5±1.11	0.47±0.01	1.93±0.02	0.26±0.01	1.51±0.01	0.96±0.05	2.47±0.06	1.59±0.08
B. mycoides	47.5±0.69	0.42±0.01	2.24±0.07	0.25±0.01	1.47±0.04	0.92±0.03	2.39±0.07	1.60±0.01
Shade+HPS								
P								
Control	25.5±0.20	0.42±0.01	1.33±0.02	0.18±0.01	1.73±0.03	1.08±0.04	2.81±0.07	1.60±0.04
B. mycoides	26.5±0.27	0.42±0.01	1.71±0.01	0.25±0.01	1.74±0.11	1.21±0.08	2.95±0.19	1.44±0.01
SGL								
Control	26.2±0.50	0.47±0.01	1.62±0.10	0.20±0.01	1.74±0.01	1.02±0.01	2.76±0.02	1.71±0.01
B. mycoides	28.3±0.20	0.42±0.01	2.00±0.07	0.28±0.01	1.57±0.01	1.04±0.03	2.61±0.02	1.52±0.05
S								
Control	20.2±0.41	0.42±0.01	0.92±0.04	0.13±0.01	1.37±0.03	0.82±0.01	2.19±0.04	1.67±0.02
B. mycoides	24.5±0.48	0.44±0.01	1.42±0.04	0.19±0.01	1.31±0.02	0.76±0.02	2.07±0.04	1.72±0.01
Sunlight								
P								
Control	56.8±0.33	0.66±0.02	5.28±0.05	0.77±0.01	1.83±0.01	1.29±0.09	3.12±0.08	1.43±0.10
B. mycoides	50.0±0.48	0.50±0.01	5.50±0.14	0.78±0.01	1.85±0.01	1.16±0.01	3.01±0.01	1.59±0.02
SGL								
Control	57.3±0.91	0.69±0.01	5.82±0.04	0.85±0.06	1.71±0.04	1.07±0.05	2.78±0.09	1.61±0.05
B. mycoides	50.8±0.58	0.53±0.01	5.82±0.07	0.83±0.02	1.65±0.01	1.10±0.05	2.75±0.05	1.51±0.06
S								
Control	55.7±0.43	0.53±0.01	5.05±0.06	0.64±0.03	1.43±0.01	0.84±0.01	2.27±0.01	1.71±0.02
B. mycoides	48.6±0.45	0.47±0.01	5.35±0.09	0.72±0.07	1.67±0.02	1.03±0.02	2.7±0.03	1.62±0.01
Shade								
P								
Control	15.5±0.53	0.41±0.01	0.56±0.01	0.06±0.01	1.43±0.08	0.83±0.05	2.26±0.14	1.74±0.01
B. mycoides	15.4±0.37	0.42±0.01	0.62±0.01	0.07±0.01	1.38±0.05	0.98±0.09	2.36±0.14	1.42±0.08
SGL								
Control	16.2±0.34	0.40±0.01	0.62±0.02	0.07±0.01	1.23±0.02	0.75±0.03	1.98±0.02	1.66±0.09
B. mycoides	18.4±0.39	0.39±0.01	0.69±0.01	0.09±0.01	1.30±0.04	0.76±0.03	2.06±0.07	1.72±0.02
S								
Control	11.6±0.35	0.40±0.01	0.42±0.01	0.05±0.01	1.13±0.07	0.70±0.03	1.82±0.09	1.62±0.04
B. mycoides	14.3±0.28	0.40±0.01	0.44±0.01	0.06±0.01	1.13±0.01	0.65±0.01	1.79±0.01	1.74±0.01
p (L × C × I)	0.023	< 0.001	0.019	0.044	0.004	< 0.001	0.001	< 0.001

DGCI: Dark Green Colour Index. Chl: Chlorophyll.

Significant three-way interactions were observed for canopy cover and DGCI (L x C x I; p = 0.023 and p < 0.001, respectively). Inoculation decreased both canopy cover and DGCI under sunlight across all cultivars, but its effects under Shade + LED and Shade + HPS were generally improved but it was cultivar-dependent (Fig. 1). The average growth rate was influenced by significant L × C (p = 0.002) and L × I (p < 0.001) interactions (Table 3 and Table 4). Cultivar S showed greater growth rate and inoculation significantly increased growth rate only under Shade + HPS (Fig. 2).

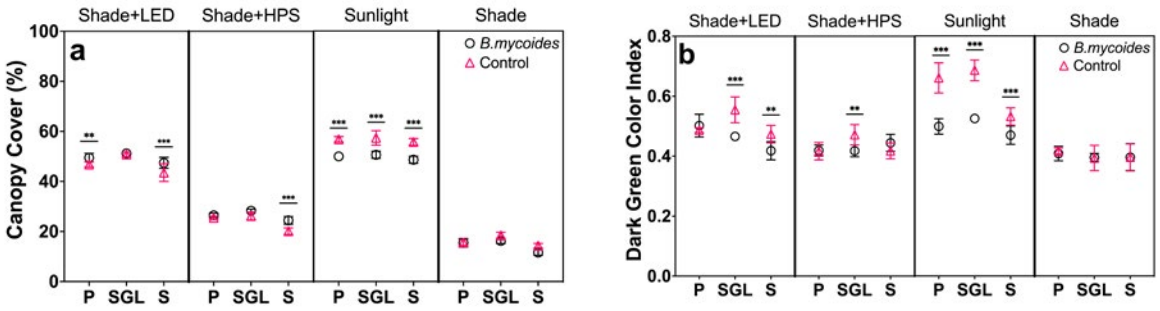

Figure 1. Effects of light environment, cultivar, and *Bacillus mycoides* inoculation on (a) canopy cover, (b) dark green colour index. Light environments: Shade+LED, Shade + HPS, Sunlight, and Shade. Cultivars: P ('Presidian'), SGL ('Apple SGL'), S ('Solstice II'). Circles represent *B. mycoides* inoculated plants; triangles represent uninoculated controls. Error bars indicate 95% confidence intervals; where not visible, error bars are smaller than the symbols. Asterisks indicate significant differences between *B. mycoides* and control treatments (Sidak post-hoc test: *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001).

Table 3. Effects of light environment (L) x cultivar (C) interactions on growth rate and root length of ryegrass cultivars (Mean ± SE, n = 4). Ryegrass cultivars, P: 'Presidian', SGL: 'Apple SGL', S: 'Solstice II'

Treatment	Growth Rate	Root Length
Unit	mm/day	cm
Shade+LED		
P	0.71±0.01	15.00±0.22
SGL	0.66±0.01	14.85±0.20
S	0.77±0.02	15.49±0.32
Shade+HPS		
P	0.62±0.03	10.88±1.25
SGL	0.63±0.02	10.87±1.24
S	0.65±0.02	11.12±1.27
Sunlight		
P	0.40±0.01	18.47±0.17
SGL	0.34±0.01	17.47±0.17
S	0.39±0.01	21.52±0.29
Shade		
P	0.32±0.01	4.88±0.42
SGL	0.32±0.01	4.32±0.25
S	0.38±0.01	6.12±0.28
p (L × C)	0.002	0.001

Table 4. Effects of light environment (L) × *Bacillus mycoides* inoculation (I) interactions on growth rate and root length of ryegrass cultivars (Mean ± SE, n = 4)

Treatment	Growth Rate	Root Length
Unit	mm/day	cm
Shade+LED		
Control	0.70±0.02	15.56±0.16
B. mycoides	0.72±0.02	14.67±0.14
Shade+HPS		
Control	0.59±0.01	8.17±0.10
B. mycoides	0.67±0.01	13.74±0.12
Sunlight		
Control	0.38±0.01	19.28±0.68
B. mycoides	0.37±0.01	19.02±0.58
Shade		
Control	0.34±0.01	5.72±0.30
B. mycoides	0.35±0.01	4.49±0.29
p (L × I)	<0.001	<0.001

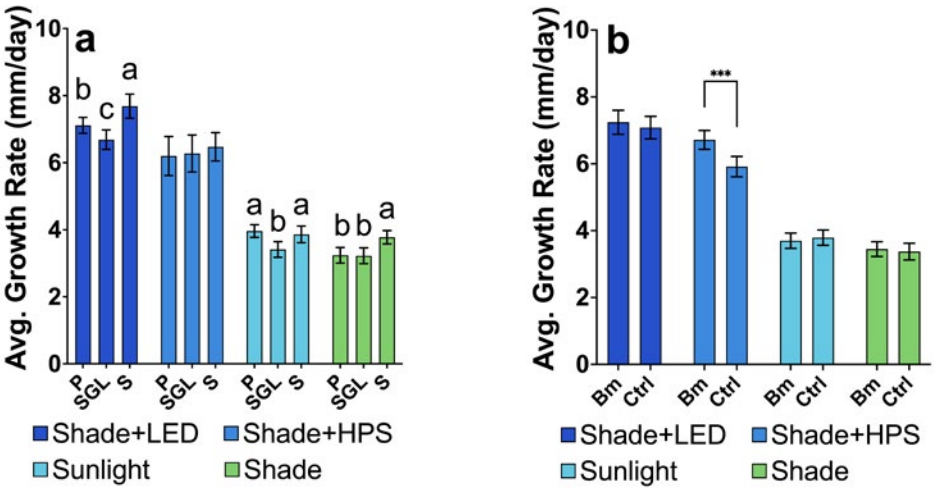

Figure 2. Effects of interactions on average growth rate: (a) light × cultivar interaction and (b) light × inoculation interaction. Light environments: Shade+LED, Shade+HPS, Sunlight, and Shade. Cultivars: P ('Presidian'), SGL ('Apple SGL'), S ('Solstice II'). Bm.: *B. mycoides*; Ctrl: Control. Error bars indicate 95% confidence intervals. Letters in (a) indicate significant differences among treatments within each light environment (Sidak, $p \leq 0.05$). Asterisks in (b) indicate significant differences between B.m. and Ctrl treatments (Sidak post-hoc test: *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$).

Root development was shaped by significant interactions. Root length varied according to L × C ($p < 0.001$) and L × I ($p < 0.001$). S had the longest root length under sunlight. Inoculation significantly increased root length only under Shade + HPS (Fig. 3). For root biomass, both fresh and dry root weights were influenced by three-way interactions (L × C × I; $p = 0.019$ and $p = 0.044$, respectively), with inoculation benefits evident under Shade + LED and Shade + HPS but absent under sunlight and Shade (Fig. 4).

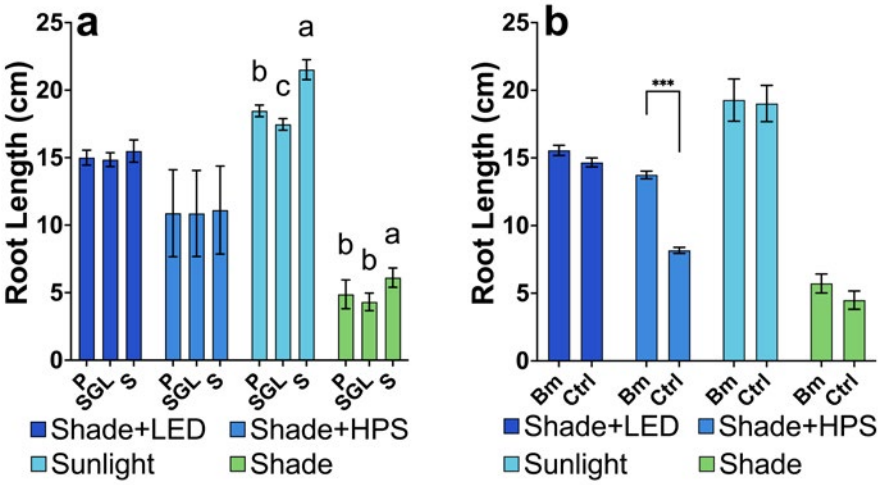

Figure 3. Effects of interactions on root length: (a) light × cultivar interaction and (b) light × inoculation interaction. Light environments: Shade+LED, Shade+HPS, Sunlight, and Shade. Cultivars: P ('Presidian'), SGL ('Apple SGL'), S ('Solstice II'). Bm.: *B. mycoides*; Ctrl: Control. Error bars indicate 95% confidence intervals. Letters in (a) indicate significant differences among treatments within each light environment (Sidak, $p \leq 0.05$). Asterisks in (b) indicate significant differences between B.m. and Ctrl treatments (Sidak post-hoc test: *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$).

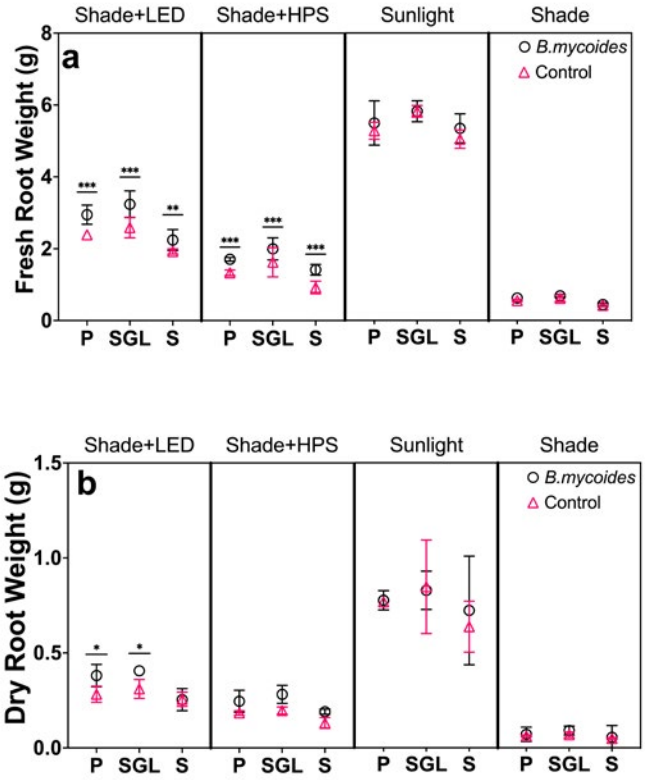

Figure 4. Effects of light environment, cultivar, and *Bacillus mycoides* inoculation on (a) fresh root weight, and (b) dry root weight. Light environments: Shade + LED, Shade + HPS, Sunlight, and Shade. Cultivars: P ('Presidian'), SGL ('Apple SGL'), S ('Solstice II'). Circles represent *B. mycoides* inoculated plants; triangles represent uninoculated controls. Error bars indicate 95% confidence intervals; where not visible, error bars are smaller than the symbols. Asterisks indicate significant differences between *B. mycoides* and control treatments (Sidak post-hoc test: *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$).

Chlorophyll parameters were governed by similar three-way interactions (L × C × I; $p = 0.004$, $p < 0.001$, $p = 0.001$ and $p < 0.001$ for chlorophyll a, b, total chlorophyll and chlorophyll a/b, respectively), indicating that inoculation impact on pigment concentration and composition was strongly dependent on both light environment and cultivar (Fig. 5). Total chlorophyll content was generally higher under LED than HPS and sunlight yielded the best results. Generally, S had the higher chlorophyll a/b ratio under different light environments. However, only cultivar P under Shade and *B. mycoides* inoculation increased chlorophyll a/b ratio.

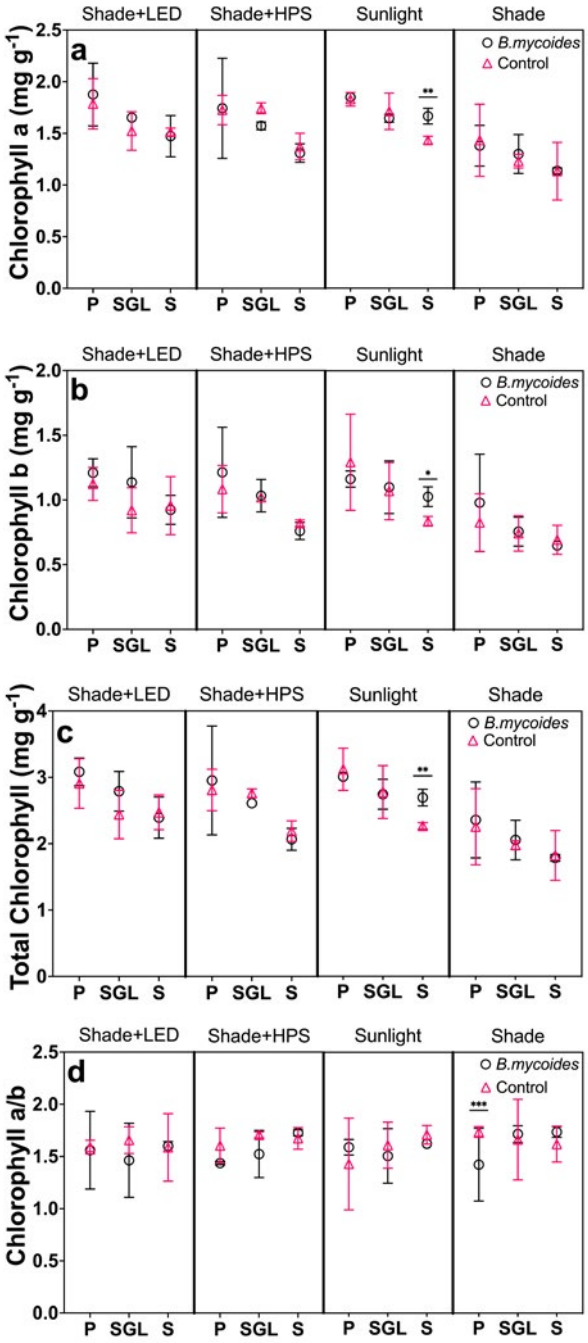

Figure 5. Effects of light environment, cultivar, and *Bacillus mycoides* inoculation on (a) chlorophyll a, (b) chlorophyll b, (c) total chlorophyll, and (d) chlorophyll a/b ratio. Light environments: Shade + LED, Shade + HPS, Sunlight, and Shade. Cultivars: P ('Presidian'), SGL ('Apple SGL'), S ('Solstice II'). Circles represent *B. mycoides* inoculated plants; triangles represent uninoculated controls. Error bars indicate 95% confidence intervals; where not visible, error bars are smaller than the symbols. Asterisks indicate significant differences between *B. mycoides* and control treatments (Sidak post-hoc test: *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001).

4. DISCUSSION

This study examined the combined effects of light environment, cultivar selection and *Bacillus mycoides* inoculation on early establishment traits in perennial ryegrass. While we hypothesized that *B. mycoides* would promote better establishment under favourable light conditions, our results revealed unexpected complexity. Contrary to expectations, *B. mycoides* inoculation reduced DGCI and canopy cover under sunlight, while supplemental lighting enhanced establishment compared to Shade as anticipated. These findings demonstrate that PGPR efficacy in turfgrass is dependent on specific lighting technologies rather than simply light availability, fundamentally altering our understanding of the plant-microbe relationship under different light environments.

4.1. Light environment effects

As expected, Shade resulted in the poorest turf performance across all measured traits. Canopy cover under Shade ranged from only 11.6-18.4% compared to 48.6-57.3% under sunlight conditions. This outcome reflects significant photosynthetically active radiation (PAR) reduction that limits carbohydrate production and triggers cellular stress responses (Turnbull, 2021; Brito et al., 2023), with detrimental shading effects on *Lolium perenne* L. varieties well documented (Dąbrowski et al., 2015; Zhang K et al., 2024). The 70% light reduction likely triggered oxidative stress through disrupted electron transport chain function, leading to reactive oxygen species accumulation in chloroplasts and reduced photosystem II efficiency (Ihtisham et al., 2023). Low PAR also induced typical shade avoidance responses including reduced tillering and leaf production, which reduce canopy cover, while promoting vertical growth attempts to reach available light (Kumar R et al., 2024; Wherley et al., 2005). This response, coupled with anatomical changes like thinner leaves and weaker cell walls (Allard et al., 1991), made the etiolated growth unsustainable, as plants became prostrate after every irrigation in the present study (Fig. 6).

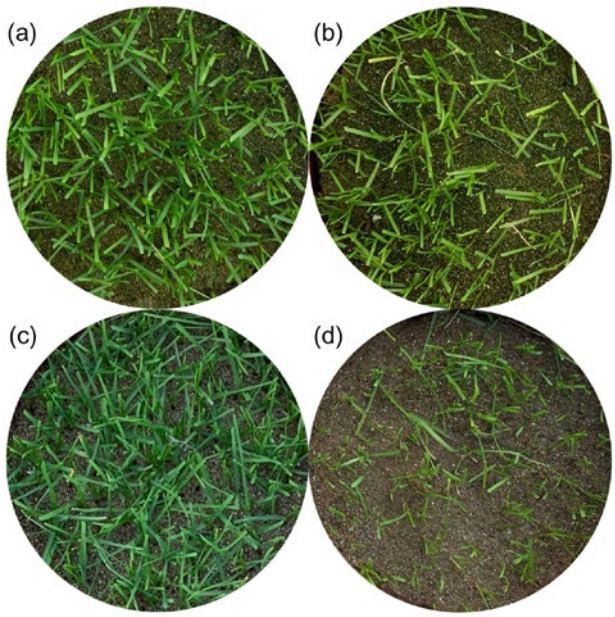

Figure 6. Representative images of ryegrass establishment under different light environments: (a) Shade + LED, (b) Shade + HPS, (c) Sunlight, and (d) Shade. Images show typical canopy development and turfgrass density under each lighting condition at the end of the experiment. All images were taken from the uninoculated control group of the 'Apple SGL' cultivar.

Supplemental LED and HPS lighting enhanced turf performance under shaded conditions compared to Shade alone, consistent with findings by Abélard & Galbrun (2022). The spectral composition of HPS lighting, relatively low in blue light and enriched in red and far-red wavelengths (Islam et al., 2012), likely activates phytochrome-mediated pathways that strongly induce shade-avoidance responses

including pronounced stem elongation and reduced tillering (Kumar R et al., 2024). In our findings, the observed increase in vertical growth, alongside reduced canopy cover suggests a shift in growth allocation. Plants appeared to prioritize shoot elongation over lateral tiller development. This response may be driven by the extended 18-hour photoperiod and specific spectral properties of the light. These conditions can influence hormone regulation. Elevated levels of auxins and gibberellins are known to promote stem elongation. At the same time, they may suppress tiller formation (Wherley et al., 2005; Yunze & Shuangsheng, 2014). In contrast, LED lighting achieved canopy cover levels only slightly below sunlight (43.5-51.2%), whereas HPS resulted in significantly lower coverage (20.2-28.3%), suggesting LED provides more balanced spectral output supporting lateral growth and tillering.

From a practical stadium management perspective, these findings have important economic implications. LED lighting not only achieved superior canopy cover compared to HPS but also offers ~40% energy savings. This dual advantage of better establishment outcomes with lower operational costs strengthens the case for LED adoption in professional sports venues.

4.2. Cultivar responses

In this study, cultivar genetics fundamentally dictated turfgrass performance. 'Solstice II' (S), a *Lolium × boucheanum* hybrid, consistently exhibited superior average growth rate and root length compared to perennial ryegrass cultivars 'Presidian' (P) and 'Apple SGL' (SGL), likely due to hybrid vigor combining rapid growth tendencies of annual ryegrass with perennial ryegrass durability (Kennedy & O'Donovan, 2014). However, this elongation advantage coincided with lower canopy cover and DGCI values compared to other cultivars, with these baseline differences influencing subsequent responses to light environments and bacterial inoculation (Fu et al., 2020).

4.3. *Bacillus mycoides* inoculation effects

The present study determined that effectiveness of *B. mycoides* inoculation varied considerably depending on environmental conditions and cultivar genetics, consistent with research indicating that plant growth-promoting microorganism responses are shaped by specific environmental and genetic contexts (Li et al., 2024; Zhang & Rue, 2024).

Under supplemental lighting conditions, inoculation demonstrated clear benefits. Canopy cover increased in S (9.2% under Shade + LED, 21.3% under Shade + HPS) and P (5.8% under Shade + LED) compared to uninoculated treatments. Fresh root weight increased significantly across all cultivars under both supplemental lighting conditions, though dry root weight effects were limited to P and SGL under Shade + LED. Under supplemental lighting conditions, *B. mycoides* inoculation significantly enhanced root biomass development. Fresh root weight increased by 23.4% in 'Presidian' under Shade + LED (2.95 vs 2.39 g), 25.1% in 'Apple SGL' under Shade+LED (3.24 vs 2.59 g), and 28.5% in 'Shade+HPS' across cultivars. Dry root weight showed similar improvements, with increases of 35.7% in 'Presidian' and 32.3% in 'Apple SGL' under Shade+LED conditions. Literature widely indicates that PGPR benefits are frequently maximized when host plants encounter abiotic stresses (Kumar D et al., 2024; Li et al., 2024).

The improved performance under artificial lighting may be attributed to several mechanisms associated with *B. mycoides*, including production of indole-3-acetic acid (IAA) through tryptophan-dependent pathways and ACC deaminase activity (Ali et al., 2022; Mesquita et al., 2023). IAA production may enhance internal auxin levels supporting root development, while ACC deaminase activity reduces ethylene accumulation, preventing premature senescence under abiotic stress conditions (Ali et al., 2022).

Notably, inoculation increased both vertical growth rate and root length only under Shade+HPS. This enhancement may result from HPS thermal radiation (Katzin et al., 2021) creating microenvironments that optimize bacterial metabolic activity, as *Bacillus* species including *B. mycoides* exhibit optimal growth at 26–33°C (Satapute et al., 2012). Although the greenhouse provided semi-controlled environmental conditions, the separated lighting systems likely created distinct thermal microenvironments, with

HPS potentially generating higher localized temperatures than LED or ambient conditions. However, canopy and root zone temperatures were not measured, limiting the ability to verify the temperature-related mechanism. Bacterial populations were also not quantified, which prevents direct evaluation of whether the observed plant responses reflect actual differences in *B. mycoides* establishment across light environments. Future studies should include both thermal monitoring and bacterial enumeration to confirm temperature-dependent effects on bacterial performance.

In contrast, inoculation under sunlight conditions reduced canopy cover by 11.3-12.8% and DGCI by 11.3-24.2% across all cultivars. Under non-stressful conditions, microbial associations may impose metabolic costs (Klein et al., 2022), diverting resources from tillering or pigment synthesis when plants are already performing optimally. This supports the principle of growth-defense tradeoffs, where diverting resources to maintain a symbiosis or a defense-primed state can reduce growth when the plant is not limited by other factors (Ku et al., 2024; Fan & Jespersen, 2025).

4.4. Physiological responses

Dark Green Colour Index and chlorophyll-related parameters were significantly affected by three-way interactions among treatments. DGCI values were generally highest under sunlight and lowest under Shade. Shifts in chlorophyll concentration suggest adjustments in photosystem stoichiometry to optimize light capture efficiency under varying spectral conditions (Lokstein et al., 2021) with blue light, for instance, playing a recognized role in chlorophyll formation (Ouzounis et al., 2015). A common response to moderate shade involves an increase in chlorophyll b-rich antenna proteins, which reduces the chlorophyll a/b ratio to enhance photon absorption (Bailey et al., 2001; Walters, 2004). However, under severe shade, this pattern was not consistently observed. In several cases, the chlorophyll a/b ratio remained stable or increased. For example, in the uninoculated P, the ratio under Shade was 1.74 compared to 1.43 under sunlight. This suggests that the imposed shade levels exceeded the threshold for adaptive responses, potentially disrupting pigment synthesis or altering photosystem composition (Kulikova et al., 2019). The typical increase in antenna size seen under moderate shade was not evident. Instead, reduced chlorophyll content and deviations from expected pigment ratios indicate a shift from acclimation to stress (Zhang L et al., 2024). This non-linear response, where increasing shade intensity leads to physiological impairment rather than adaptation, has been reported in other turfgrass species as well (Walton et al., 2025; Wang et al., 2024). Overall, these findings support the view that turfgrass responses to low light conditions may transition from adaptive to detrimental once critical light thresholds are crossed.

Early establishment of perennial ryegrass is shaped by complex interactions among light environment, cultivar genetics, and microbial inoculation, with significant three-way interactions dominating most responses. Shade consistently yielded the poorest performance across all parameters, while LED supplemental lighting facilitated canopy cover approaching Sunlight levels. In contrast, HPS lighting, despite promoting vertical elongation, resulted in significantly thin canopies, likely due to its spectral output inducing stronger shade-avoidance morphology.

Contrary to expectations, *B. mycoides* inoculation reduced canopy cover (11.3-12.8%) and DGCI (11.3-24.2%) under sunlight conditions, indicating potential metabolic costs under non-stressful conditions. Conversely, under supplemental lighting, inoculation enhanced canopy cover (5.8-21.3%) and root development, suggesting effective stress mitigation. These findings demonstrate that PGPR efficacy in turfgrass is dependent on specific lighting technologies rather than simply light availability.

From a practical stadium management perspective, LED lighting not only achieved superior canopy cover compared to HPS, but also offers ~40% energy savings, strengthening the case for LED adoption in professional sports venues. The conditional nature of *B. mycoides* benefits suggests PGPR applications should target specific stress conditions rather than universal application.

Supplementary information ↑

Funding sources

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Supplementary material

Not applicable.

Data availability

The data supporting the findings of this study are available from the corresponding author, Ertuğrul Balekoğlu, upon reasonable request.

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Authorship contribution statement

Ertuğrul Balekoğlu: Conceptualization, Methodology, Investigation, Formal Analysis, Writing - Original Draft Preparation, Writing - Review & Editing, Project Administration.

Ali Salman: Conceptualization, Methodology, Investigation, Resources, Writing - Review & Editing, Supervision.

Bülent Yağmur: Conceptualization, Investigation, Resources, Writing - Review & Editing, Supervision.

Competing interests

The authors declare that Global Growlight Firm and Akademi Tohum Company, which provided materials (lighting equipment and turfgrass seeds, respectively) free of charge for this study, had no role in the study design, data collection, data analysis, data interpretation, or manuscript preparation. The authors declare no other competing interests.

Statement on the use of Artificial Intelligence

The author(s) employed ChatGPT (OpenAI, GPT-5, 2026) in order to translate the Title, Abstract, and Keywords from English into Spanish while preparing this work. Following their use, the author(s) assumed full responsibility for the publication's content and reviewed and edited it as necessary.

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