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Enhancing maize growth and reducing irrigation needs with extracellular polymeric substances and microbial inoculants

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ABSTRACT

Soil amendments and microbial inoculants can affect plant growth, water retention, and crop resilience. This study investigated the effects of two amendments, extracellular polymeric substances (EPS) and biochar, with and without bacterial inoculation, on maize (*Zea mays*) growth, irrigation needs, and physiological responses. Maize was cultivated in soil with 2.5 % and 5 % (w/w) of wet EPS (Kaumera®) or biochar and inoculated with a bacterial consortium consisting of *Arthrobacter nicotinovorans* EAPPA and *Rhodococcus* sp. EC35.

EPS-treated plants exhibited significantly higher shoot biomass, larger stem thickness, while soil plant analysis development (SPAD) values suggest improved nutrient availability and photosynthetic efficiency. In non-inoculated plants, EPS supplementation increased shoot dry biomass by 78 % and stem thickness by 9 % compared to control plants grown without amendments. This enhancement strongly correlated with nutrient uptake, especially in plants supplemented with 5 % of EPS. Particularly, Mg and Ca concentrations increased by 195 % and 73 %, respectively, compared to non-amended controls. Inoculation further amplified these benefits, underscoring its key role in plant development and resilience. In contrast, biochar-treated plants exhibited reduced growth, suggesting stress effects at the tested addition doses. Electrolyte leakage, a key indicator of plant stress, was significantly lower in soils amended with EPS, suggesting that EPS provides a protective effect to the plants. EPS also demonstrated remarkable water retention benefits, reducing irrigation requirements by 30 % with 5 % of EPS application, compared to 9 % reduction with biochar. The use of EPS, combined with microbial inoculants, represents a sustainable agricultural strategy for optimizing maize production in water-limited environments.

1. Introduction

Climate change poses a significant challenge for global agricultural production, jeopardizing the ability to meet the increasing food demands of a growing population expected to reach 10 billion by 2050 (Kumar et al., 2021; Yuan et al., 2024). Among its consequences, drought stands out as a major threat, severely reducing crop yields and disrupting farming systems, while exerting substantial pressure on already scarce water resources. It not only impacts immediate agricultural productivity but also endangers its long-term sustainability (Muzammal et al., 2024).

Maize (*Zea mays* L.) is one of the world's most extensively cultivated food crops playing a crucial role in the food provision of billions of people (Erenstein et al., 2022). However, maize production is highly vulnerable to water shortages, with numerous studies highlighting the adverse effects of drought on its performance. Széles et al. (2023) reported that maize grown under dry and extremely dry field conditions, had lower soil plant analysis development (SPAD) and leaf area index values, along with accelerated chlorophyll degradation and leaf senescence. Similarly, Sah et al. (2020) reported that water deficiency during

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pre-flowering and grain-filling stages significantly affected maize, particularly in non-drought-tolerant lines. Drought delayed flowering and maturity, increased anthesis-silk intervals, reduced leaf numbers, and disrupted root development, leading to severe yield losses.

Over the past decade, substantial efforts have been made to mitigate the impact of water scarcity on maize growth, with a particular focus on the use of plant growth-promoting bacteria (PGPB) (Pereira et al., 2020; Saleem et al., 2021; Shirinbayan et al., 2019). Beneficial microorganisms play a crucial role in enhancing plant resilience to drought stress through multiple mechanisms, like the production of phytohormones, which promote root elongation and branching, enabling plants to explore deeper soil layers for water and nutrients. Additionally, PGPB enhance drought tolerance by promoting osmotic adjustments through the production of osmolytes such as proline and trehalose, boosting antioxidant activity, and regulating stress-responsive gene expression (Chieb and Gachomo, 2023; El-Saadony et al., 2024). The use of soil water retainers, such as hydrogels, biopolymers, and organic amendments, has also emerged as an effective strategy for mitigating drought stress (Kang et al., 2022; Miller and Naeth, 2019). For instance, biochar application has been shown to improve soil structure by enhancing aggregation, reducing bulk density, and increasing soil water use efficiency (Razzaghi et al., 2020). Despite numerous studies investigating the use of EPS-producing bacteria as biofertilizers (Costa et al., 2018; Naseem et al., 2024), the practical application of extracted EPS as soil amendment in agriculture remains limited. EPS have shown great promise in enhancing soil health and agricultural productivity. By facilitating the formation of stable soil aggregates and improving overall soil structure, EPS contribute to increased water retention, enhanced nutrient availability, and reduced vulnerability to erosion (Saha et al., 2020). A key innovation of this work lies in the novel application of extracellular polymeric substances (EPS) - commercially known as Kaumera® - extracted from aerobic granular sludge from a full-scale wastewater treatment plant, as a soil amendment. Sludge-derived EPS are typically a by-product of wastewater treatment and are often underutilized or discarded (Hamed et al., 2025). By exploring their use in agriculture, this research introduces a new valorization pathway for these biopolymers, transforming a waste stream into a value-added product with potential benefits for soil health and plant resilience.

The primary objective of this study was to investigate the effects of combining plant growth-promoting bacteria (PGPB) with different doses of soil amendments - biochar and EPS - on maize growth and water irrigation requirements. By using EPS as soil amendment, this study also seeks to evaluate a sustainable approach that optimizes the use of an underutilized resource, while enhancing maize growth.

2. Materials and methods

2.1. Bacterial strains

Two bacterial strains from the ESB-CBQF collection were selected for this study: *Arthrobacter nicotinovorans* EAPPA and *Rhodococcus* sp. EC35. These strains were originally isolated from a metal-contaminated site in the North of Portugal (Pires et al., 2017). Both strains exhibit strong plant growth-promoting traits, including phosphorus (P) solubilization, high production of indole-3-acetic acid (IAA) and siderophores, as well as ACC-deaminase activity (Pereira and Castro, 2014). Additionally, previous research has demonstrated their ability to enhance maize growth under various stress conditions (Moreira et al., 2016; Pereira et al., 2020).

To ensure culture purity, bacterial strains were streaked onto Trypticase Soy Agar (TSA) medium and incubated at 30 °C for 24 h to ensure culture purity. Following this, biocompatibility between strains was assessed on TSA plates. Each bacterial strain was suspended in 10 mL of Trypticase Soy Broth (TSB) and incubated at 30 °C overnight at room temperature. For the biocompatibility test, one strain was streaked across the TSA plate and then droplets of the second strain were applied. Plates were incubated at 30 °C for 3 days, with observations recorded at 24h and 48h.

2.2. Greenhouse experimental design

A two-month greenhouse pot experiment was conducted to evaluate the effects of EPS, biochar, and PGPB inoculation on maize growth and irrigation requirements. The greenhouse conditions were carefully controlled, maintaining a 12 h photoperiod with 450 μmol m^{-2} s^{-1} photosynthetically active radiation, a temperature range of 18–21 °C, and relative humidity between 50 and 60 %. The soil used in this study was randomly collected from an agricultural area in northern Portugal and classified as sandy loam. It had a pH of 6.5 (H_2O) and 5.8 ($CaCl_2$), an electric conductivity of 0.148 mS cm^{-1}, and an organic matter content of 5.72 %. The total nitrogen content was 0.27 %. Regarding extractable nutrient levels, the soil contained 2581 mg kg^{-1} of P_2O_5, 122 mg kg^{-1} of K_2O, 3452 mg kg^{-1} of CaO, 134 mg kg^{-1} of MgO, 19.4 mg kg^{-1} of S, and 0.588 mg kg^{-1} of B. Chemical fertilization was not applied, as the soil already contains sufficient levels of essential nutrients to effectively support maize growth.

The experiment followed a factorial design with the following treatments: non-amended soil as the control (C), soil amended with 2.5 % (w/w) wet EPS (2.5 % EPS), soil amended with 5 % (w/w) wet EPS (5 % EPS), soil amended with 2.5 % (w/w) Biochar (2.5 % B), and soil amended with 5 % (w/w) Biochar (5 % B). Each treatment was applied both with and without PGPB inoculation. All treatments were replicated four times. EPS and biochar were manually mixed with soil, and each mixture was distributed into 2 kg pots.

EPS in the form of Kaumera® (w/w) and biochar (w/w) were incorporated into the soils one day prior seed transplantation. Kaumera® (https://kaumera.com/english/) is a bio-based material extracted from aerobic granular sludge formed during the Nereda® wastewater purification process. It was obtained from a mobile demonstration plant operated by the TUDelft at WWTP Utrecht, the Netherlands (https://shorturl.at/OQdmQ). Kaumera exhibited the following chemical properties: total carbohydrates 168 mg g^{-1} volatile solids, total proteins 323 mg g^{-1} volatile solids, pH 2.3, electrical conductivity 10.1 mS cm^{-1}, total C 40.2 % of total solids, total N 6.01 % of total solids, total K 28.18 mg g^{-1} dry matter (DM), total P 20.76 mg g^{-1} DM, total Ca 4.7 mg g^{-1} DM (Posada, 2023). The biochar (Ecochar®) was sourced from Ibero Massa Florestal, S.A., Portugal (Ecochar® Technical Sheet, 2024) and exhibited the following properties: particle size of 1–10 mm, pH range of 8–10, total fixed C $\geq$ 90 %, total N $\leq$ 5 g kg^{-1}, total Cd $<$ 0.05 mg kg^{-1}, total Pb 0.05 mg kg^{-1}, total Fe 99.5 mg kg^{-1}, and total As and Hg $<$ 0.01 mg kg^{-1}. Additionally, it contained $\leq$5 % ash, $<$30 % humidity, and $<$5 % volatile matter (Arrobas et al., 2022; Martins et al., 2022). This biochar was produced in a pyrolytic reactor using wood biomass derived from silver wattle (*Acacia dealbata*) (Arrobas et al., 2022).

Maize seeds (*Zea mays* cv. DKC 5110, cycle 400) were sterilized with 0.5 % (v/v) NaOCl for 30 min and rinsed several times with deionized sterile water. Three seeds were transferred to plastic pots containing 2 kg of sieved (2 mm) non-amended and amended (with EPS and B) agricultural soil. For inoculation, 50 mL of a bacterial inoculum containing equal proportions of both strains were sprayed onto the soil surface one week after seedling emergence. To prepare the inoculum, both bacterial strains were grown in TSB at 30 °C with agitation at 120 rpm until reaching an inoculum density of ca. 10^8 colony forming units (CFU) mL^{-1}. In the control pots (without bacterial inoculation), 50 mL of diluted TSB medium (1:3) was applied. Three weeks after planting, the seedlings were thinned to two plants per pot. The pots were randomly distributed in the greenhouse, a process repeated weekly during the experiment.

Soil moisture content was determined gravimetrically by weighing fresh soil samples (collected twice weekly), oven-drying them at 105 °C for 24h, and reweighing. Soil moisture content (%) was calculated as:

(Wet weight – Dry weight)/Dry weight × 100. Based on these measurements, the amount of water added to each pot was calculated to maintain moisture content at 25 % during the first four weeks. Previous results showed that this moisture level provides adequate conditions for early maize development while preventing waterlogging in a sandy loam soil. In the remaining weeks, the moisture content was increased to 35 % to meet the higher water demands of maize at later growth stages. After 8 weeks, plants were harvested and washed with deionized water to remove soil/dust residues, and biometric parameters were determined.

2.3. *Soil plant analysis development (SPAD) and biometric parameters*

Before harvest, the total chlorophyll index was determined using an indirect Chlorophyll Meter (SPAD 502 Plus, Spectrum Technologies, Inc.), which assesses light absorption by the leaf, following the method outlined by Rocha et al. (2022). Measurements were taken in three positions along the first fully developed leaf: approximately 10 cm from the tip, in the midpoint, and 10 cm from the base. Stem width was measured with a digital caliper (Mitutoyo) 25 cm above the base of the stem for both plants in each pot. Plant height was recorded from the base to the tip of the uppermost leaf. Root and shoot dry biomass were determined after drying for 1 week at 65 °C.

2.4. *Analysis of shoot nutrient content*

Oven-dried shoots were finely ground, and 0.5 g of each sample was used for chemical digestion with 10 mL of HNO_3 (Sigma-Aldrich). A blank digestion was prepared with 10 mL of HNO_3 for reference. Four biological replicates were used for each treatment. The volume of the digested samples was adjusted to 20 mL using ultra-pure water and stored at 4 °C until analysis. The digested solutions were filtered using a disposable syringe filter (Chromafil Pet - 45/25) for subsequent analysis. Nutrient content, including Na, Mg, Ca, P, and K, was analyzed using the Optima 7000 DV Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (PerkinElmer, USA).

2.5. *Electrolyte leakage index*

Cell membrane integrity damage was assessed using the electrolyte leakage (EL) method (Rocha et al., 2022). Briefly, at harvest, 10 leaf discs (5 from each plant) were collected from the first fully developed leaf and placed in a tube with 20 mL of deionized water. The tubes were left at room temperature for 2h. After this period, the initial electrical conductivity (EC1) of the solution was measured using a conductivity meter (Multi 340i digital meter, WTW). The samples were then autoclaved at 121 °C for 20 min. Once cooled, a second electrical conductivity reading (EC2) was taken. The EL was calculated using the following formula:

$$EL = EC1/EC2 \times 100$$

2.6. *Statistical analysis*

Statistical analyses were performed using the statistical software package SPSS 26.0 (SPSS Inc., Chicago, IL, USA) and R (version 4.3.3). Two-way ANOVA was performed to assess the significant differences in the effect of soil amendments (EPS and biochar) and bacterial inoculation on each parameter. One-way ANOVA with Duncan post hoc analysis was also performed to assess the effects of soil amendments on the different plant parameters for non-inoculated and inoculated plants.

Pearson correlation matrix and the significance levels were performed using the R function *rcorr* () from the package *Hmisc*. A correlogram with significance levels was created with the function *corrplot* () from the *corrplot* package. Principal component analysis (PCA) was performed using the R function *pca* () from the *FactoMineR* package, while its visualization was generated by using the *fviz_pca_biplot* () function from the *factoextra* package in R.

3. Results and discussion

3.1. *Maize growth response to soil amendments and microbial inoculants*

The effect of soil amendments and bacterial inoculants on the growth of maize is shown in Fig. 1. No significant differences ($p > 0.05$) were observed in root dry biomass across treatments (Fig. 1B). However, soil supplementation with EPS at both doses (2.5 and 5 %) significantly increased shoot dry biomass and stem thickness of non-inoculated plants by approximately 78 % and 9 %, respectively compared to the control plants (Fig. 1A and C). A slight increase (+5 %) in shoot elongation was also observed for plants growing with 5 % of EPS (Fig. 1B). Indeed, EPS-containing amendments are known to enhance soil aggregation and nutrient retention, which can indirectly promote plant growth by improving soil structure, fertility, and water-holding capacity (Costa et al., 2018; Paul et al., 2024; Saha et al., 2020). In addition, numerous studies highlighted the beneficial effects of EPS-producing bacteria on crop development, reporting increases in plant biomass and yield (Dar et al., 2021; Khan and Bano, 2019; Saha et al., 2020). Aoudi et al. (2024) also demonstrated that EPS extracted and purified from the bacterial strain *Enterobacter ludwigii* significantly improved rice shoot and root dry weights by 14 % and 27 %, respectively, under drought stress conditions. In the present study, bacterial inoculation further enhanced maize growth, increasing shoot dry biomass by 37 % and stem thickness by 15 % compared to plants grown in non-amended and non-inoculated soil. Furthermore, inoculated plants grown in soil amended with 5 % of EPS showed greater improvements, with shoot dry biomass increasing by 50 % and stem thickness by 18 % compared to inoculated plants in non-amended soil. The strains used in this study, *A. nicotinovorans* EAPAA and *Rhodococcus* sp. EC35, have previously shown significant positive effects on maize growth (Pereira and Castro, 2014). This is likely due to their strong production of IAA, which enhances root development and nutrient uptake, as well as their ability to solubilize P and produce siderophores. Similarly, Naseem et al. (2024) and Dartora et al. (2016) reported that PGPB inoculation effectively increased maize growth. In addition, the synergetic beneficial effects of EPS and PGPB may be attributed to EPS enhancing the effectiveness of bacterial strains by sustaining their survival in soil, as they improve nutrient retention, acting as an alternative energy source, facilitating nitrogen acquisition, and promoting microbial cross-feeding and cooperation (Zhang et al., 2024).

In contrast with EPS outcomes, shoot dry biomass and stem thickness declined with the application of 2.5 % and 5 % of biochar, suggesting a detrimental effect of this amendment on plant growth. This negative impact may be attributed to toxic substances present in biochar, including polycyclic aromatic hydrocarbons, polychlorinated dibenzo-p-dioxins, furans, and volatile organic compounds, and/or changes on soil properties such as pH and electrical conductivity (Brtnicky et al., 2021). Bai et al. (2022) reported a decline in the germination rate, shoot and root length of maize with increasing biochar application rates, with the effect becoming more pronounced at higher concentrations (100, 200, and 300 g/L). However, in the present work, bacterial inoculation notably alleviated the negative effects of biochar on maize development, restoring stem thickness and shoot dry biomass to levels similar to those of the control. Godinho et al. (2025) also reported similar results, noting that while the application of comparable biochar doses to contaminated soil reduced sunflower growth, the use of bacterial inoculants effectively mitigated these negative effects.

3.2. *Nutritional benefits of soil amendments and microbial inoculants in maize*

The impact of soil amendments and microbial inoculants on the

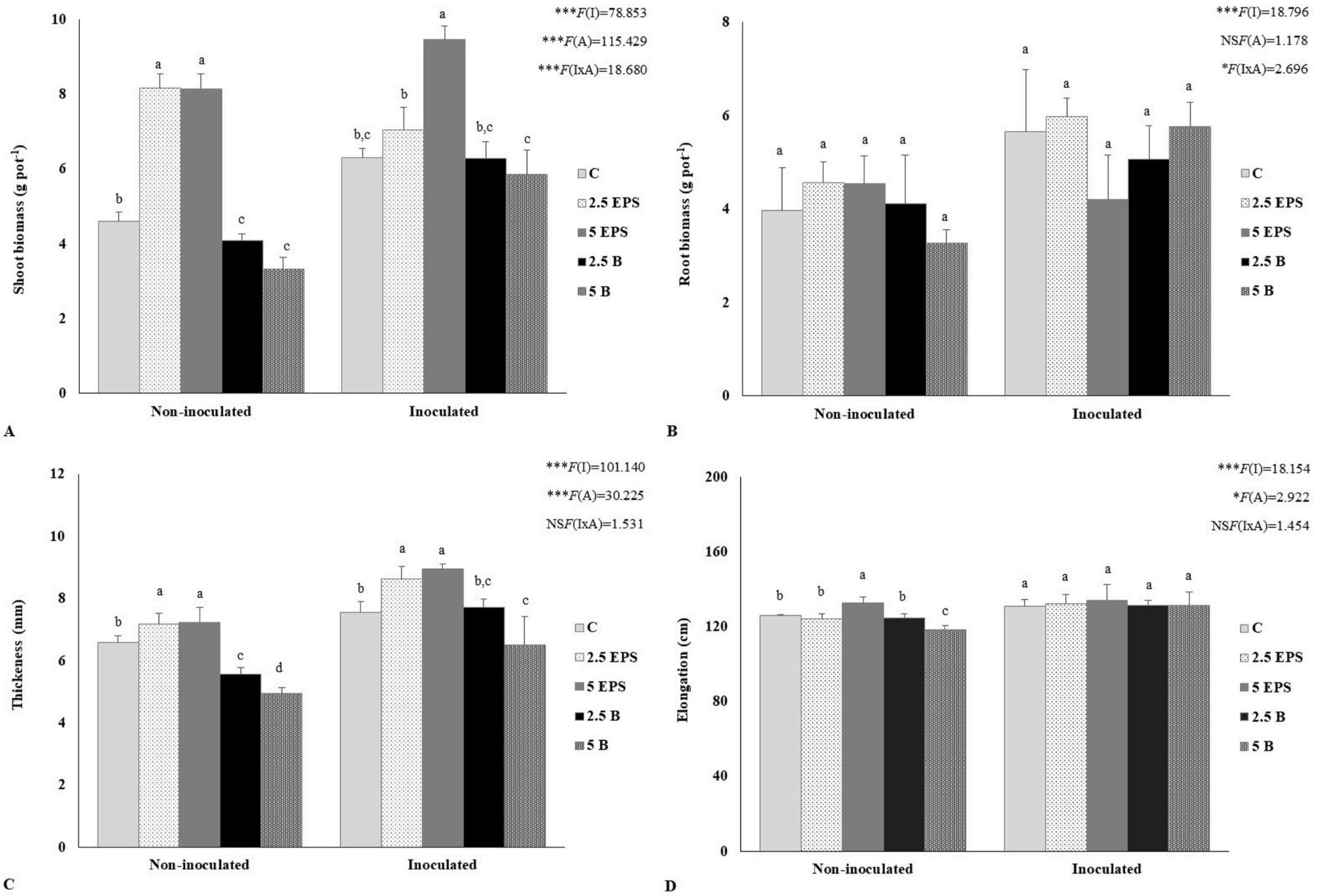

Fig. 1. Shoot (A) and root (B) dry biomass, shoot thickness (C) and shoot elongation (D) of maize plants grown in non-amended soil (C) and in soil amended with different doses (2.5 and 5 %) of EPS and Biochar (B) under non-inoculated and inoculated conditions at the end of the experiment.
Values are means ± standard deviation (n = 4). A two-way ANOVA was performed to determine the influence of inoculation (I) and amendments (A) on shoot and root biomass, shoot thickness and shoot elongation of maize plants. The results are shown with the test statistic for each case (I: inoculation; A: amendments; I x A: inoculation x inoculation) and as NS: Non-significant at the level $p > 0.05$; *significant at the level $p < 0.05$; **significant at the level $p < 0.01$; ***significant at the level $p < 0.001$, respectively. One-way ANOVA was performed to determine the influence of amendments (EPS and B) on shoot and root dry biomass, shoot thickness and shoot elongation for non-inoculated and inoculated conditions. Means for the same inoculation condition showing different letters are significantly different ($p < 0.05$) from each other according to Duncan test. For shoot dry biomass, the F values of one-way ANOVA are ***$F = 165.916$ and ***$F = 26.586$, respectively for non-inoculated and inoculated conditions. For root dry biomass, the F values of one-way ANOVA are NS$F = 1.602$ and NS$F = 2.178$, respectively for non-inoculated and inoculated conditions. For shoot thickness, the F values of one-way ANOVA are ***$F = 32.049$ and ***$F = 9.644$, respectively for non-inoculated and inoculated conditions. For shoot elongation, the F values of one-way ANOVA are ***$F = 14.278$ and NS$F = 0.151$, respectively for non-inoculated and inoculated conditions.

nutrient content of maize shoots is presented in Table 1. In non-inoculated plants, the addition of 5 % of EPS significantly enhanced the concentrations of P, Mg, Ca, and Na in maize shoots. Notably, the increases in Mg and Ca reached 195 % and 73 %, respectively, compared to control plants without amendments. A similar pattern was observed in inoculated plants, where both nutrient concentrations showed a further increase compared to non-amended plants.

These findings are consistent with Paul et al. (2024), who reported that microbial EPS can capture and retain nutrients. The presence of hydroxyl and carboxyl groups gives EPS a negative charge, granting them a high cationic exchange ability, allowing an effective binding of essential nutrients and enhancing their availability for plant uptake.

The increased concentration of nutrients in maize shoots aligns with the enhanced growth observed in plants cultivated in soils amended with 5 % of EPS. This relationship is further supported by PCA analysis (Fig. 2), where the first two principal components account for 61.9 % of the total variance. Notably, Mg and Ca exhibited a strong correlation with key growth parameters, including elongation, stem thickness, and shoot biomass, underscoring their crucial role in plant development (Fig. 2; Supplementary material_1). Moreover, EPS treatments, in both inoculated and non-inoculated plants, are predominantly clustered on the right side of the PCA, aligning with the vectors representing plant growth parameters, reinforcing the positive impact of EPS treatments on maize growth (Fig. 2). According to Zhang et al. (2024), EPS can enhance nutrient and water utilization by both microorganisms and plants, ultimately benefiting the soil-microbe-plant system. However, direct evidence supporting the role of EPS as a soil amendment to improve plant nutrient uptake remains limited. According to Table 1, the addition of biochar, particularly at 5 %, also significantly increased the concentration of certain nutrients in maize shoots. This effect was most notable for P, K, and Na in inoculated plants. However, these nutrient increases do not correlate positively with growth parameters (Table 1; Fig. 2).

3.3. *Physiological responses of maize to soil amendments and microbial inoculants*

The SPAD readings, which reflect chlorophyll content and overall plant health, varied significantly across treatments in both non-inoculated and inoculated plants (Table 2). Non-amended plants (C)

Table 1
Nutrient content in shoots of maize plants grown in non-amended soil (C) and in soil amended with different doses (2.5 and 5 %) of EPS and Biochar (B) under non-inoculated and inoculated conditions at the end of the experiment.

Treatments		P (mg kg-1)	Mg (mg kg-1)	Ca (mg kg-1)	K (mg kg-1)	Na (mg kg-1)
Non-inoculated	**C**	4697.7 ± 131.5^{c}	1807.2 ± 30.8^{c}	5748.5 ± 164.9^{b}	3959.9 ± 199.4^{b}	85.1 ± 12.1^{b,c}
	2.5 EPS	4406.4 ± 338.3^{c}	2228.2 ± 206.6^{b}	6186.9 ± 860.9^{b}	3162.2 ± 151.7^{d}	78.8 ± 5.7^{c}
	5 EPS	6277.5 ± 591.1^{a}	5348.5 ± 286.6^{a}	9923.0 ± 893.6^{a}	3501.1 ± 109.8^{c}	102.7 ± 14.1^{a}
	2.5 B	4607.7 ± 316.0^{c}	2144.4 ± 152.5^{b,c}	6049.3 ± 223.2^{b}	4114.5 ± 91.6^{b}	78.6 ± 13.9^{c}
	5 B	5455.4 ± 119.0^{b}	1827.2 ± 176.8^{c}	5588.4 ± 874.4^{b}	5284.1 ± 295.3^{a}	96.0 ± 5.9^{b,c}
		***F = 14.237	***F = 189.265	***F = 21.442	***F = 57.537	*F = 2.854
Inoculated	**C**	4687.3 ± 298.8^{b}	3397.5 ± 279.3^{b}	7675.3 ± 456.0^{b}	3366.1 ± 62.0b	200.8 ± 45.6^{b}
	2.5 EPS	5022.2 ± 618.7^{b}	2866.1 ± 543.8^{b}	6350.5 ± 1023.5^{c}	3316.3 ± 296.3b,c	129.6 ± 10.7^{b,c}
	5 EPS	4952.3 ± 385.9^{b}	4993.0 ± 373.9^{a}	9981.2 ± 245.2^{a}	3009.9 ± 177.8c	121.0 ± 9.4^{c}
	2.5 B	4448.4 ± 255.6^{b}	2734.5 ± 132.1^{b,c}	6585.3 ± 643.5^{c}	3169.7 ± 139.4b,c	161.9 ± 35.6^{b,c}
	5 B	6066.9 ± 200.2^{a}	2263.0 ± 109.1^{c}	5697.0 ± 141.8^{c}	5393.9 ± 134.5a	367.4 ± 70.4^{a}
		***F = 7.934	***F = 30.722	***F = 24.251	***F = 90.364	***F = 17.893
		NSF(I) = 0.158 ***F(A) = 14.769 ***F(IxA) = 6.961	***F(I) = 34.855 ***F(A) = 130.551 ***F(IxA) = 9.956	*F(I) = 5.834 ***F(A) = 43.043 NSF(IxA) = 2.274	**F(I) = 28.255 ***F(A) = 136.767 ***F(IxA) = 10.177	***F(I) = 95.816 ***F(A) = 17.877 ***F(IxA) = 15.908

Values are means ± standard deviation (n = 4). A two-way ANOVA was performed to determine the influence of amendments (A) and inoculation (I) on nutrient content in shoots. The results are shown with the test statistic for each case (I: inoculation; A: amendment; I x A: inoculation x amendments) and as NS: Non-significant at the level $p > 0.05$; *significant at the level $p < 0.05$; **significant at the level $p < 0.01$; ***significant at the level $p < 0.001$, respectively. One-way ANOVA was performed to determine the influence of amendments (EPS and B) on nutrient content in shoots for non-inoculated and inoculated conditions. Means for the same inoculation condition showing different letters are significantly different from each other ($p < 0.05$) according to Duncan test.

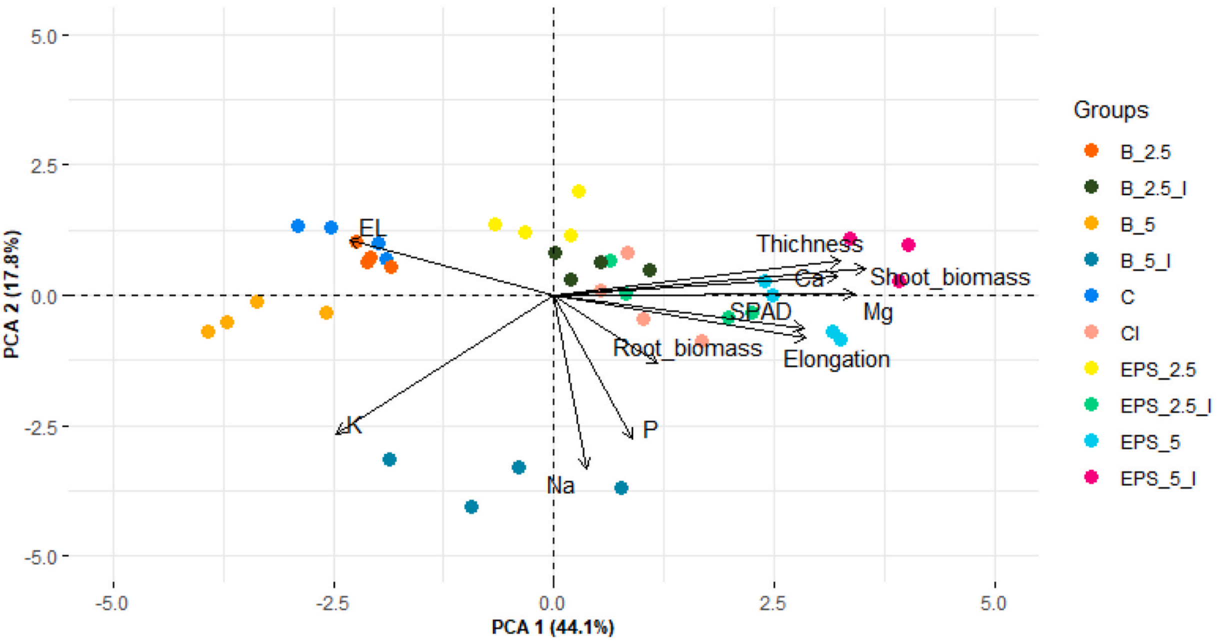

Fig. 2. Principal component analysis (PCA) biplot showing the relationship between the different treatments and growth, nutritional and physiological plant parameters. The arrows represent the contribution of each variable to the PCA dimensions, with longer arrows indicating stronger influences.
C: control, non-inoculated; CI: control, inoculated; EPS_2.5: 2.5 % EPS-amended soil, non-inoculated; EPS_2.5_I: 2.5 % EPS-amended soil, inoculated; EPS_5: 2.5 % EPS-amended soil, non-inoculated; EPS_5_I: 5 % EPS-amended soil, inoculated; B_2.5: 2.5 % biochar-amended soil, non-inoculated; B_2.5_I: 2.5 % biochar-amended soil, inoculated; B_5: 5 % biochar-amended soil, non-inoculated; B_5_I: 5 % biochar-amended soil, inoculated. EL: electrolyte leakage.

showed the lowest SPAD values in non-inoculated plants (20.7 ± 6.1), but inoculation led to a notable increase (+48 %) of this parameter. This aligns with Ojuederie and Babalola (2023), who also reported that microbial inoculation increased chlorophyll content in maize. Soil supplementation with 5 % of EPS significantly increased SPAD values by 90 % in non-inoculated and by 29 % in inoculated plants, indicating a strong positive effect of EPS on chlorophyll content. A similar, though less pronounced trend was observed with 2.5 % of EPS supplementation, particularly in non-inoculated plants. The highest SPAD values were recorded in plants grown in 5 % of EPS-amended soils, which also exhibited increased Mg accumulation in shoots. This aligns with the strong correlation between these two parameters observed in the PCA (Fig. 2) and in the correlogram (Supplementary material_1). Indeed, Mg is a key component of the chlorophyll molecule, playing a crucial role in photosynthesis, enzyme activation, and nutrient transport (Ishfaq et al., 2022). Biochar treatments at both 2.5 % and 5 % led to moderate increases in SPAD value in non-inoculated plants, whereas in inoculated plants, no significant improvements were observed (Table 2).

The effect of soil amendments and inoculation on EL of maize leaves is presented in Fig. 3. In non-inoculated plants, EL was significantly higher in non-amended and biochar-amended treatments. PCA further supports this, showing that some biochar-treated samples align with EL (Fig. 2), suggesting a stress response that likely contributed to the reduced plant growth observed in biochar-amended plants. A

Table 2

SPAD readings of leaves of maize plants grown in non-amended soil (C) and in soil amended with different doses (2.5 and 5 %) of EPS and Biochar (B) under non-inoculated and inoculated conditions at the end of the experiment (harvest).

Treatments	SPAD	
	Non-inoculated	Inoculated
C	20.7 ± 6.1^{d}	30.5 ± 2.2^{b}
2.5 EPS	$23.8 \pm 1.3^{c,d}$	31.5 ± 1.6^{b}
5 EPS	39.3 ± 2.8^{a}	39.6 ± 1.3^{a}
2.5 B	30.7 ± 3.7^{b}	29.6 ± 3.1^{b}
5 B	$27.3 \pm 2.2^{b,c}$	28.3 ± 4.0^{b}
	$^{***}F = 15.770$	$^{***}F = 9.867$
$^{**}F(\mathrm{I}) = 11.819$; $^{***}F(\mathrm{A}) = 22.488$; $^{***}F(\mathrm{IxA}) = 4.559$		

Values are means ± standard deviation (n = 4). A two-way ANOVA was performed to determine the influence of amendments (A) and inoculation (I) on chlorophyll content. The results are shown with the test statistic for each case (I: inoculation; A: amendment; I x A: inoculation x amendments) and as NS: Non-significant at the level $p > 0.05$; *significant at the level $p < 0.05$; **significant at the level $p < 0.01$; ***significant at the level $p < 0.001$, respectively. One-way ANOVA was performed to determine the influence of amendments (EPS and B) on SPAD readings for non-inoculated and inoculated conditions. Means for the same inoculation condition showing different letters are significantly different from each other ($p < 0.05$) according to Duncan test.

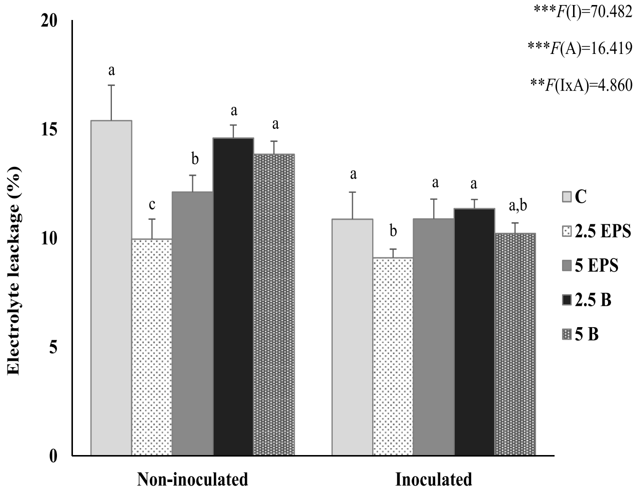

Fig. 3. Leaf electrolyte leakage (EL) of maize plants grown in non-amended soil (C) and in soil amended with different doses (2.5 and 5 %) of EPS and Biochar (B) under non-inoculated and inoculated conditions at the end of the experiment.

Values are means ± standard deviation (n = 4). A two-way ANOVA was performed to determine the influence of inoculation (I) and amendments (A) on EL of maize plants. The results are shown with the test statistic for each case (I: inoculation; A: amendments; I x A: inoculation x inoculation) and as NS: Non-significant at the level $p > 0.05$; *significant at the level $p < 0.05$; **significant at the level $p < 0.01$; ***significant at the level $p < 0.001$, respectively. One-way ANOVA was performed to determine the influence of amendments (EPS and B) on EL for non-inoculated and inoculated conditions. Means for the same inoculation condition showing different letters are significantly different ($p < 0.05$) from each other according to Duncan test. The F values of one-way ANOVA are $^{***}F = 14.749$ and $^{*}F = 3.913$, respectively for non-inoculated and inoculated conditions.

contrasting trend was observed in Abbas et al. (2024), where the application of biochar combined with compost and animal manure significantly reduced EL in maize leaves. Similarly, Liu et al. (2024) reported that biochar application lowered EL in *Melissa officinalis*, suggesting its potential role in mitigating oxidative stress and improving membrane stability. In this study, plants grown in 2.5 and 5 % EPS-amended soils exhibited the lowest EL values, indicating a protective effect. This finding is reinforced by the negative correlation between EL and growth parameters (Supplementary material_1), suggesting that improved plant growth and physiological attributes are associated with lower EL values (Fig. 2). In inoculated plants, soil amendments had a marginal effect, with consistently lower EL values across all treatments, suggesting that bacterial inoculation mitigates membrane damage and enhances plant resilience. This aligns with Romero-Munar et al. (2023), who reported that microbial inoculation significantly reduced EL, improving maize performance under heat and drought stress.

The total water applied to all pots throughout the experiment is presented in Fig. 4. Inoculation did not significantly affect maize irrigation requirements, whereas the addition of soil amendments had a notable impact on water usage. Specifically, the addition of 5 % of EPS reduced irrigation needs by c.a. 30 %, while biochar application led to only a 9 % reduction. These findings suggest that EPS, particularly at higher application rates, enhances soil water retention, making it a promising amendment for improving water efficiency in agriculture. The ability of EPS to retain water is well-documented in the literature. Certain EPS molecules, such as xanthan gum, have been reported to exhibit an exceptionally high water-holding capacity (Costa et al., 2018), helping to mitigate drought stress by retaining moisture in the rhizosphere. Additionally, EPS function as natural binding agents, forming bridges between soil particles and clay, which enhances soil structure and minimizes water loss.

4. Conclusions

This study highlights the significant benefits of EPS derived from wastewater treatment sludge and microbial inoculants in improving maize growth, reducing irrigation needs and enhancing plant resilience. EPS-treated plants exhibited increased shoot biomass, thickness, and chlorophyl content (SPAD values), indicating improved nutrient availability and photosynthetic efficiency. In contrast, although biochar slightly reduced irrigation needs, it appeared to trigger stress effects in plants, leading to reduced growth.

These findings highlight the synergetic use of EPS and microbial inoculants as a promising sustainable strategy for optimizing maize production in water-limited environments. By enhancing soil water retention and nutrient uptake, this approach offers a viable solution for improving crop performance under challenging agricultural conditions.

To fully unlock the agricultural potential of EPS, further research is needed, particularly field-based studies to validate the observed effects under real-world conditions. A deeper understanding of the mechanisms underlying plant responses to EPS is also essential.

While this study demonstrated significant improvements in plant growth, physiological performance and reduced irrigation needs, the economic viability of using EPS at the tested doses remains uncertain. Further studies should be conducted including agricultural trials to assess additional benefits such as soil erosion prevention and the potential market values of EPS. These studies should also assess the effectiveness of lower application rates and their implications for economic feasibility. If reduced rates prove less effective, EPS-based products still hold potential in high-value production systems or niche applications, such as horticulture or precision agriculture in water-limited environments, where the benefits may justify the cost. Future work should also explore the feasibility of direct sludge application as a simpler and potentially more cost-effective alternative. Overall, this study opens promising avenues for the circular reuse of wastewater by-products, contributing to both plant productivity and environmental sustainability.

CRediT authorship contribution statement

Alexandra Overall: Writing – review & editing, Methodology, Investigation, Formal analysis. **Helena Moreira:** Writing – review &

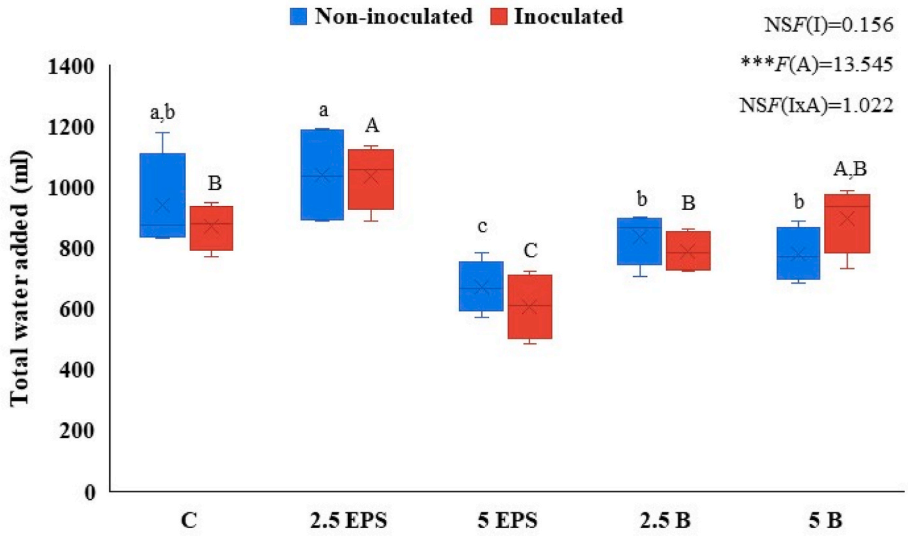

Fig. 4. Total water applied (mL) over the course of the experiment in non-amended soil (C) and in soil amended with different doses (2.5 and 5 %) of EPS and Biochar (B) under non-inoculated and inoculated conditions at the end of the experiment.

Values are means ± standard deviation ($n = 4$). One-way ANOVA was performed to determine the influence of amendments (EPS and B) on the amount of water added for non-inoculated and inoculated conditions. Means with different lowercase letters indicate significant differences ($p < 0.05$) among non-inoculated plants, while different uppercase letters indicate significant differences among inoculated plants, based on Duncan's test. The *F* values of one-way ANOVA are $**F = 5.232$ and $***F = 10.714$, respectively for non-inoculated and inoculated conditions.

editing, Supervision, Conceptualization. **Ana S.S. Sousa:** Writing – review & editing, Methodology, Formal analysis. **Philipp Wilfert:** Writing – review & editing. **Mark van Loosdrecht:** Writing – review & editing. **Paula M.L. Castro:** Writing – review & editing, Supervision, Funding acquisition. **Sofia I.A. Pereira:** Writing – review & editing, Writing – original draft, Supervision, Formal analysis, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rhisph.2025.101136.

Data availability

Data will be made available on request.

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