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Seed and Soil Inoculation With a *Bacillus* Consortium in Durum Wheat. Part I: Soil Phosphorus Content and Plant Morphological and Physiological Performance

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ABSTRACT

Reducing synthetic fertilizer inputs in durum wheat while maintaining crop performance is a key challenge for Mediterranean cereal systems, where soil constraints frequently limit nutrient availability and canopy function. This study evaluated the physiological and soil-nutrient responses of durum wheat (*Triticum turgidum* subsp. *durum* cv. Antalis) to a multi-strain *Bacillus* consortium (*B. licheniformis*, *B. methylotrophicus*, *B. megaterium*) applied through different delivery strategies under fertilized and unfertilized field conditions. Field trials were conducted during the 2023–2024 season at two contrasting Sicilian sites (Campo Carboj and Sparacia) using a randomized complete block design with three replicates. Treatments included seed coating, soil spraying at three rates and combined seed + soil applications. Post-harvest soil available P (Olsen, expressed as P_2O_5) and plant traits were assessed, alongside physiological indicators of canopy function (SPAD chlorophyll index, stomatal conductance [G_s] and NDVI across key BBCH stages). Across both sites, microbial inoculation increased soil P_2O_5 availability, with the strongest responses at Sparacia (clay-rich, more P-immobilizing conditions), where several microbial treatments produced increases comparable to mineral fertilization and, in some cases, exceeded +50% relative to the unfertilized control. Microbial application also enhanced crop physiology, particularly at Sparacia: non-fertilized treatments increased plant height (up to +23%), sustained higher SPAD values, improved G_s and increased NDVI from tillering through grain filling, indicating improved canopy vigour under nutrient limitation. Responses at Campo Carboj were generally smaller but consistently positive. Combined seed + soil applications (notably S6–S7) provided the most robust improvements, supporting the agronomic value of multi-modal inoculation strategies. Overall, the consortium partially compensated for fertilizer withdrawal by improving soil P availability and sustaining physiological *performance*, with the magnitude varying by site and driven by edaphic context.

1 | Introduction

Durum wheat (*Triticum turgidum* L. subsp. *durum* Desf) remains a strategic crop for Mediterranean agri-food systems, but its productivity is still widely supported by mineral fertilization. In parallel, EU policy frameworks (i.e., Farm to Fork and Green Deal) explicitly push agriculture to reduce nutrient losses by at least 50% and overall fertilizer use by at least 20% by

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2030, while avoiding deterioration of soil fertility—making nutrient-use efficiency a central target for cereal-based rotations (European Commission n.d.; European Commission 2020).

Among macronutrients, phosphorus (P) is a persistent constraint in many Mediterranean soils (Oliverio et al. 2020; Sardans and Peñuelas 2004). Under alkaline conditions and in soils with relevant carbonate content and reactive mineral surfaces, both native and applied P can be rapidly transformed into sparingly soluble Ca–P phases or strongly retained, reducing fertilizer efficiency and increasing the risk of chronic P limitation (Geng et al. 2022; Su et al. 2023). This issue becomes particularly relevant when soils differ markedly in texture and in carbonate-related reactions, because P diffusion and availability are strongly governed by soil physicochemical conditions (Brownrigg et al. 2022; von Wandruszka 2006). Microbial fertilization—via plant growth-promoting rhizobacteria (PGPR), phosphate-solubilizing microorganisms (PSM) and microbial consortia—has gained momentum as a complementary strategy to improve nutrient availability while reducing dependence on synthetic inputs (Etesami and Adl 2020; De Andrade Rodrigues Severo et al. 2023). Mechanistically, PSM can increase P availability through the secretion of organic acids and chelating compounds, the production of phosphatases and other enzymes involved in P mineralization and rhizosphere chemical modulation that shifts P sorption–desorption equilibria (Pang et al. 2024). Within this functional space, *Bacillus* spp. are among the most widely developed inoculants due to formulation stability (spore-formers), rhizosphere competence and multi-trait plant growth promotion (Blake et al. 2020). Recent evidence in wheat systems confirms that phosphate-solubilizing *Bacillus* strains can enhance soil biochemical functioning linked to P cycling and improve wheat growth under P-limited conditions (Iqbal et al. 2024).

Nevertheless, translating these mechanisms into consistent field-level outcomes remains non-trivial. The performance of microbial inoculants under open-field conditions is often characterized by high variability, driven by the complex interactions between the introduced strains, the fluctuating environmental factors and the resident soil microbiota (Janati et al. 2023; Korir et al. 2024; Z. Wang et al. 2021). This inconsistency represents one of the primary barriers to the large-scale adoption of bio-fertilizers. Consequently, there is a practical need for field studies that go beyond 'inoculated versus not inoculated' comparisons by testing application strategies (e.g., seed treatment vs. soil application vs. combined approaches), and by linking soil nutrient changes to in-season physiological indicators that explain *when* and *how* microbial inputs compensate for reduced fertilization (O'Callaghan et al. 2022). From an agronomic perspective, the integration of physiological and canopy proxies, such as chlorophyll indices and normalized difference vegetation index (NDVI), is increasingly used to assess crop functional status and potential productivity in durum wheat under semi-arid conditions (Tortorici et al. 2025; Rufaioğlu et al. 2025).

Within this framework, the present study evaluates a multi-strain *Bacillus* consortium (*Bacillus licheniformis*, *Bacillus methylotrophicus*, *Bacillus megaterium*) under Mediterranean field conditions, explicitly addressing context-dependency by testing two contrasting Sicilian sites and comparing seed coating, soil spraying at increasing doses and combined seed + soil inoculation under both fertilized and unfertilized management. The experimental design was guided by three central hypotheses: (i) that the integration of different delivery strategies (seed coating combined with soil drenching) would provide a more robust and stable microbial establishment compared to single-mode applications, (ii) that the effectiveness of the Bacillus consortium in mobilizing P would be strictly modulated by the soil's physical-chemical matrix, showing a more pronounced impact in the clayey-calcareous environment where P-fixation is higher and (iii) that the microbial-driven increase in P-availability would translate into measurable physiological and canopy-level enhancements (e.g., higher Gs, SPAD and NDVI), potentially compensating for the reduction or absence of mineral fertilization. By coupling post-harvest available P (Olsen P expressed as P_2O_5) with agronomic traits and in-season physiological measurements (SPAD, stomatal conductance [G_s], NDVI across key phenological stages), the study is positioned to resolve—at least in part—the main bottlenecks for adoption: (i) robustness across environments, (ii) practical optimization of delivery strategy and (iii) mechanistic interpretation of 'fertilizer replacement' via measurable soil and plant functional responses.

2 | Materials and Methods

2.1 | Experimental Sites

The study was conducted during the winter–spring growing season of 2023–2024 at two experimental farms: 'Campo Carboj', located in Castelvetrano (TP, Sicily, Italy; 37° 51′ 17″ N, 12° 53′ 40″ E; 30 m a.s.l.), and 'Sparacia', located in Cammarata (AG, Sicily, Italy; 37° 38′ 08.8″ N, 13° 45′ 46.4″ E; 390 m a.s.l.). Both sites are owned by the Regional Agency for Agricultural Development (Ente di Sviluppo Agricolo della Regione Sicilia) and are managed by the Department of Agricultural, Food and Forest Sciences of the University of Palermo. The physicochemical properties of the soils at the two experimental locations are reported in Table S1. At both sites, the preceding land use was fallow.

Soil sampling was carried out at both sites prior to sowing to establish the baseline physical and chemical characteristics of the experimental areas. For each plot, three primary samples were collected at a depth of 0–40 cm using a manual soil auger. To account for local spatial variability, these primary samples were pooled and thoroughly homogenized to obtain a single representative composite sample for each experimental unit. The resulting material was then air-dried at room temperature and sieved through a 2 mm mesh to remove coarse fragments and organic debris before being subjected to laboratory analysis.

2.2 | Plant Material and Crop Management

The crop used in this study was the durum wheat variety Antalis (Semìa) of *T. turgidum* subsp. *durum*. This medium–early cultivar is recognized for its stable agronomic performance and high yield potential. It is well-suited to both traditional and marginal cereal-growing environments in Italy due to its

resilience and tolerance to major phytopathogens. The cultivar also exhibits desirable technological quality traits—particularly gluten characteristics—essential for the milling and pasta industry.

Sowing density was set at 350 plants m^{-2}, and sowing was carried out at both experimental sites on 11 December 2023. Sowing was performed in rows using a plot seeder, with a constant inter-row spacing of 17 cm. The seeds were placed at an average depth of 4 cm to ensure optimal soil contact and uniform emergence across all experimental plots. The wheat was sown under rainfed conditions following the first consistent autumn rainfall events. Although soil water status was not directly monitored, the pluviometric regime recorded during the sowing period (November) indicates adequate moisture availability for crop establishment, consistent with typical Mediterranean clinical patterns where sowing follows the transition from the summer dry period to the humid winter season. Fields were managed according to standard traditional agricultural practices commonly adopted in Sicily, including autumn ploughing to 0.30 m followed by shallow seedbed preparation before sowing. Weed control was performed at the three-leaf stage using Diflufenican (2.2%) and Chlortoluron (35.5%) at a rate of 3.5 L ha^{-1}. Fungicide applications were conducted between late tillering and early stem elongation using Pyraclostrobin at 0.7 L ha^{-1}. Harvest took place on 15 June 2024 at both experimental locations.

2.3 | Study Set-Up and Experimental Design

The microbial consortium, composed of *B. licheniformis* (1.0×10^8 CFU g^{-1}), *B. methylotrophicus* (1.0×10^8 CFU g^{-1}) and *B. megaterium* (1.0×10^8 CFU g^{-1}) ($Glyss_{sp}$, Hello Nature, Biondrate, No, Italy), was applied using two different methods: seed coating at a rate of 0.1 kg q^{-1} of seed (S1), and soil spraying immediately after sowing. To ensure uniform distribution and optimal adherence of the inoculum to the seed coat, the microorganisms were suspended in 1 L of distilled water (equivalent to 1% w/v of the total seed weight) and applied using a pressurized spray system followed by thorough mechanical mixing. The treated seeds were then briefly air-dried to prevent premature imbibition before being loaded into the seed drill. For soil spraying, three application rates of the microbial complex were tested: 1 kg ha^{-1} (S2), 2 kg ha^{-1} (S3) and 3 kg ha^{-1} (S4). In addition, combined treatments consisting of seed coating plus soil spraying were evaluated: S1 + S2 (S5), S1 + S3 (S6) and S1 + S4 (S7). Soil spraying was carried out using 300 L ha^{-1} of water applied with a sprayer operating at 250 kPa. The control treatment (S0) consisted of soil spraying with water only. The microbial consortium was tested under both fertilized (F) and unfertilized (noF) conditions. Fertilized treatments received 165 kg ha^{-1} of diammonium phosphate (18–46–0) and 200 kg ha^{-1} of potassium sulfate (0–0–50) at sowing, followed by 190 kg ha^{-1} of urea (46–0–0) applied at the late tillering/early stem elongation stage.

The experiment was arranged in a randomized complete block design with three replicates. Each plot measured 9 m^2 and was separated from adjacent plots by a 1 m buffer. Fertilized plots were spaced 3 m apart from unfertilized plots to avoid cross-contamination.

2.4 | Meteorological Data

Meteorological data were collected from an on-site ATMOS 41 weather station (METER Group, Pullman, WA, USA). ATMOS 41 measures 12 meteorological variables, including air temperature, relative humidity, vapour pressure, barometric pressure, wind speed and direction, solar radiation, precipitation and lightning. The station was connected to a ZL6 datalogger (METER Group, Pullman, WA, USA), specifically designed to collect data from environmental sensors. The datalogger transfers data to the Cloud via a Subscriber Identity Module. Its operation is powered by six NiMH batteries charged by solar cells.

The two farms show different thermal regimes and water-availability regimes (Figure S1). The comparative analysis of the thermo-pluviometric regimes between the Campo Carboj (Figure S1A) and Sparacia (Figure S1B) sites highlights a marked Mediterranean seasonality, albeit with substantial differences in the intensity of the observed meteorological phenomena. At the Carboj site, minimum temperatures consistently remain above freezing, with summer heat peaks exceeding 40°C in August. This thermal profile is accompanied by a regular but moderately intense precipitation distribution, resulting in an estimated cumulative rainfall of approximately 210 mm over the study period. In contrast, the Sparacia site exhibits more pronounced continental characteristics, featuring a harsher winter thermal regime where, in some instances between January and February, minimum temperatures drop below 0°C. Although the summer thermal trend is slightly less extreme than at Carboj, Sparacia records pluviometric events of greater magnitude, particularly during the late winter quarter, reaching a total cumulative precipitation of nearly 480 mm. Both sites experience a period of severe water stress starting in June, when the near-total absence of rainfall coincides with rising maximum temperatures; however, this deficit appears potentially more critical at Carboj due to the lower total meteoric input and higher peak temperatures, which enhance evapotranspiration rates.

2.5 | Determination of Assimilable P After Harvest

In each experimental plot, immediately after harvest, a 100 g soil sample was collected from the 0–0.30 m depth to determine available P. Available P was quantified using the Olsen colourimetric method (Olsen et al. 1954), based on extraction with sodium bicarbonate at pH 8.5. P concentration in the extract was measured colourimetrically using a Shimadzu UVmini-1240 spectrophotometer (Shimadzu Italia srl, Milan, Italy). Results were expressed as P_2O_5 in mg kg^{-1}.

2.6 | Agronomic and Physiological Data

At the end of the flowering stage (BBCH 69) (Lancashire et al. 1991), a random sample of 25 plants per replicate was used to record the number of tillers per plant, plant height (cm) and the number of fertile spikelets per spike.

To assess the crop's physiological status, three parameters were monitored: leaf chlorophyll content, G_s and the NDVI. Leaf

chlorophyll content was measured using a Minolta SPAD-502 chlorophyll meter (Konica Minolta, Italy) on 20 fully expanded leaves per replicate, and results were expressed in SPAD units. G_s was determined with a porometer (AP4, Delta-T Devices, Cambridge, UK) on five leaves selected from three randomly selected plants within each replicate, with values expressed as mmol m^{-2} s^{-1}. NDVI was assessed using the RapidScan CS-45 active canopy sensor (Holland Scientific, Lincoln, NE, USA). Measurements were taken by scanning the canopy at 1 m above the soil surface, maintaining a constant speed and a fixed 90° angle relative to the crop. Each plot was fully scanned, and NDVI values were computed automatically by the instrument. Chlorophyll content and NDVI measurements were performed at BBCH growth stages 29, 39 and 69 (Zadoks et al. 1974), whereas G_s was measured at BBCH stages 29 and 39.

2.7 | Statistical Analysis

All analyses were carried out using Minitab Statistical Software (version 21.3; Minitab LLC, State College, PA, USA). Data were subjected to analysis of variance (ANOVA) using a randomized complete block design. The assumptions of normality and homogeneity of variance were verified before analysis. When significant effects were detected ($p \leq 0.05$), mean separation was performed using Tukey's Honest Significant Difference (HSD) post hoc test. Statistical procedures followed the methodology described by Gomez and Gomez (1984).

3 | Results

3.1 | Soil Available P

A significant effect of microbial application (A), fertilization (F) and their interaction (A×F) on soil available P_2O_5 was observed at both experimental sites (Table S2).

At Sparacia, the highest P_2O_5 concentrations were observed under the S1, S5, S6 and S7 treatments, at 38.4, 35.3, 37.3 and 38.7 mg kg^{-1}, respectively. The control (S0) showed the lowest value (23.0 mg kg^{-1}), although values for S2 and S3 were not statistically different. Similarly, at Carboj, S7 recorded the highest concentration (29.4 mg kg^{-1}), whereas S2 and S0 had the lowest values (22.7–23.9 mg kg^{-1}). Across both sites, fertilization significantly increased soil P_2O_5, with a + 42% increase in Sparacia and a + 52% in Carboj compared to unfertilized plots ($p < 0.001$; Table 1). Considering the interaction effect (Figure 1), all microbial treatments combined with fertilization led to the highest P increases in Sparacia, except for S0, S2 and S3, whereas S0 under non-fertilized conditions showed the lowest P increases ($p < 0.001$; Figure 1). At Carboj, the interaction effect was milder, with S6 × F and S7 × F exhibiting the highest values (33.74 and 35.39 mg kg^{-1}, respectively); however, they were not statistically different from the other fertilized treatments, as all were grouped into the same statistical category 'a' in the post hoc test (Figure 1). Beyond these effects, a closer evaluation of the unfertilized treatments revealed that several microbial solutions also produced notable increases relative to the unfertilized control (S0 × noF). At Sparacia, intermediate microbial applications such as S2 × noF, S3 × noF, S5 × noF and S6 × noF improved soil P_2O_5 by approximately +11%, +18%, +39% and +51%, respectively, while higher-intensity treatments (S1, S4, S7) achieved increases exceeding +50% (up to +63%). At Carboj, although the magnitude of the response was smaller, intermediate solutions such as S3 × no fert, S5 × no fert and S6 × noF still produced appreciable increases (+19% to +48%) over the unfertilized control.

TABLE 1 | Soil available phosphorus (P_2O_5) concentration at the two Sicilian sites (Sparacia and Campo Carboj) as affected by application treatment and fertilization.

	Concentration on P_2O_5 in the soil	
Treatments	**Sparacia (mg kg^{-1})**	**Carboj (mg kg^{-1})**
Application (A)		
S0	23.03 ± 1.65 b	23.91 ± 3.94 b
S1	38.37 ± 3.77 a	25.53 ± 1.72 ab
S2	25.46 ± 1.29 b	22.70 ± 2.85 b
S3	23.38 ± 1.20 b	24.12 ± 2.60 ab
S4	31.66 ± 3.84 ab	24.21 ± 2.78 ab
S5	35.33 ± 3.59 a	24.87 ± 0.810 ab
S6	37.32 ± 4.01 a	27.84 ± 2.74 ab
S7	38.72 ± 3.35 a	29.43 ± 2.89 a
p value	0.000	0.006
Fertilization (F)		
No fertilization (noF)	26.91 ± 1.03 b	20.14 ± 0.682 b
Fertilization (F)	38.27 ± 2.06 a	30.52 ± 0.839 a
p value	0.000	0.000

Note: Means and standard errors are reported. Within each column, values with different letters are significantly different at $p \leq 0.05$, according to Tukey's HSD test. S0, soil spraying with water only; S1, seed coating at a dose of 100 g q^{-1} of seeds; S2, soil spraying after sowing at a dose of 1 kg ha^{-1} of microbial complex; S3, soil spraying after sowing at a dose of 2 kg ha^{-1} of microbial complex; S4, soil spraying after sowing at a dose of 3 kg ha^{-1} of microbial complex; S5, S1 + S2; S6, S1 + S3; S7, S1 + S4.

3.2 | Agronomical Traits

ANOVA revealed that the factors application (A), fertilization (F) and their interactions showed significant effects in several cases, as observed in Table S2.

At Sparacia, S7 produced the tallest plants (61.9 ± 2.7 cm), while S0 resulted in the shortest (51.8 ± 3.2 cm) ($p < 0.001$; Table S2). A comparable trend was observed at Carboj, although with generally lower values (48.8 ± 0.6 vs. 44.1 ± 0.2 cm for S7 and S0, respectively). Fertilization markedly increased plant height at Sparacia (+10.0 cm; $p < 0.001$), whereas no significant effect was detected at Carboj ($p = 0.477$) (Table S3).

The interaction between microbial application and fertilization significantly influenced plant height at both sites ($p = 0.01$ at Sparacia; $p = 0.04$ at Carboj) (Table S2; Figure S2), producing distinct response patterns. At Sparacia, plant height ranged from 45.40 cm in S0 × noF to 67.93 cm in S7 × F. Under non-fertilized

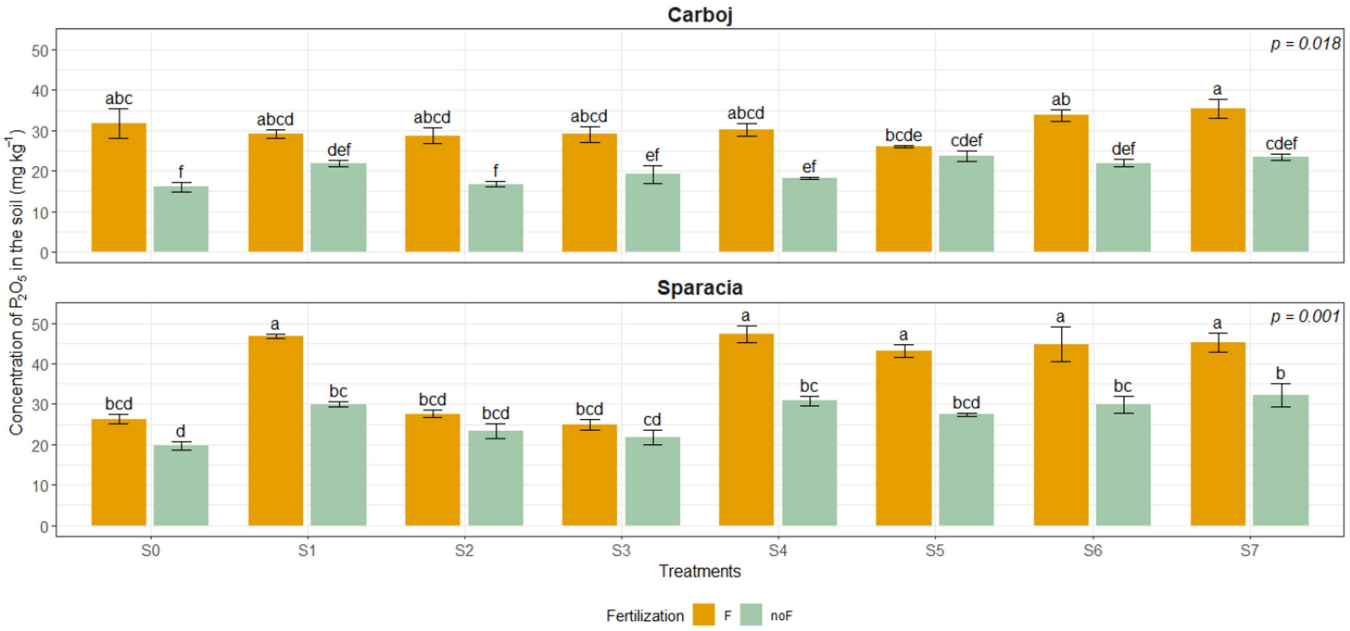


FIGURE 1 | Influence of the application × fertilization interaction on soil phosphorus content at the two Sicilian sites (Sparacia and Campo Carboj). For each data series, values with different letters are significantly different at $p \leq 0.05$, according to Tukey's HSD test. Error bars represent standard error. F, fertilization; noF, unfertilized; S0, soil spraying with water only; S1, seed coating at a dose of 100 g q^{-1} of seeds; S2, soil spraying after sowing at a dose of 1 kg ha^{-1} of microbial complex; S3, soil spraying after sowing at a dose of 2 kg ha^{-1} of microbial complex; S4, soil spraying after sowing at a dose of 3 kg ha^{-1} of microbial complex; S5, S1 + S2; S6, S1 + S3; S7, S1 + S4.

conditions, the lowest values were consistently recorded for the control, whereas combinations of microbial application with fertilization—particularly S7 × F, S4 × F and S3 × F—resulted in the highest plant heights. Overall, fertilized plots displayed clearly greater plant height than their non-fertilized counterparts, with increases becoming progressively stronger as microbial application intensity increased. At Carboj, plant height exhibited a narrower range (43.76–49.13 cm). Yet, the pattern was similar: the highest values occurred in fertilized treatments combined with microbial inoculation (e.g., S1 × F, S3 × F, S4 × F, S7 × F), while the lowest were associated with unfertilized control and low-intensity microbial applications (S0 × noF and S2 × noF). In both environments, unfertilized conditions showed that microbial applications alone improved plant height relative to the unfertilized control (S0 × noF). In fact, at Sparacia, intermediate microbial treatments (e.g., S3 × noF, S6 × noF, S7 × noF) increased height by approximately 16%–23%, and even moderate solutions such as S2 × noF and S5 × noF produced measurable gains. Whereas, at Carboj, although overall variability was reduced, treatments S6 × no fert and S7 × noF still achieved modest but consistent improvements over the unfertilized control, indicating that microbial inoculation exerted a positive effect even in the absence of mineral fertilization.

The effects of application on tiller number were not significant at Sparacia, while a slight yet significant effect was observed at Carboj ($p = 0.05$) (Table S2). Fertilization increased tillering only at Carboj ($p = 0.001$), where S3–S7 tended to show higher values (Table S3).

The number of fertile spikelets per spike did not significantly respond to microbial application (A) at either experimental site ($p = 0.614$ at Sparacia; $p = 0.845$ at Carboj) (Table S2). In fact, for both environments, despite these small numerical increases in inoculated treatments, the lack of statistical significance suggests that spikelet fertility remained relatively stable, regardless of microbial inoculation strategy. Unlike the application factor, fertilization exerted a significant positive effect on the number of fertile spikelets at Sparacia ($p = 0.009$) but not at Carboj ($p = 0.206$). Under fertilization, spikelets increased from 15.86 (unfertilized) to 17.07 (fertilized), suggesting that nutrient availability—rather than microbial inoculation—was the main driver of spikelet fertility in the heavier clay soil (Table S3).

3.3 | Physiological Parameters

The repeated measures analysis of SPAD for application (A), fertilization (F) and their interaction A×F is shown in Table S4.

In the Sparacia field, statistical differences regarding application were found only on survey dates 39 and 69 of the BBCH scale, with the highest value recorded in the S7 application method. Regarding fertilization, statistical differences were found in phases 29 and 69 of the BBCH scale, with the fertilized treatments showing consistently higher values. In Campo Carboj, application showed no statistical differences, while fertilization in phases 29 and 69 of the BBCH scale. In this case, the fertilized treatments showed the highest values (Table 2).

S7 × F consistently recorded the highest SPAD values on each of the three assessment dates (averages of 51.6, 55.5 and 58.13, respectively). During the first measurement, the other fertilized treatments did not differ statistically from S7 × F, whereas differences became more pronounced by the final measurement (Figure 2). The lowest average SPAD values were recorded for S7× noF at BBCH29 (40.36 ± 2.02) and, eventually, for S0 × noF

TABLE 2 | SPAD index (unitless) measured at three wheat growth stages (BBCH 29, BBCH 39 and BBCH 69) at the two Sicilian sites (Sparacia and Campo Carboj) as affected by application treatment (A: S0–S7) and fertilization (F: no fertilization vs. fertilization).

	SPAD BBCH 29		SPAD BBCH 39		SPAD BBCH 69	
Treatments	**Sparacia**	**Carboj**	**Sparacia**	**Carboj**	**Sparacia**	**Carboj**
Application (A)						
S0	47.35 ± 1.16 a	43.98 ± 0.49 a	47.51 ± 1.29 c	53.66 ± 0.57 a	46.43 ± 0.68 d	52.11 ± 1.19 a
S1	48.48 ± 1.02 a	42.66 ± 0.54 a	51.46 ± 0.83 ab	53.06 ± 0.49 a	52.81 ± 1.16 ab	51.01 ± 0.64 a
S2	48.88 ± 0.82 a	43.93 ± 0.55 a	49.85 ± 1.12 bc	54.66 ± 0.55 a	49.11 ± 0.86 cd	52.03 ± 1.40 a
S3	49.56 ± 0.74 a	44.48 ± 0.91 a	50.13 ± 0.72 bc	52.98 ± 0.59 a	50.10 ± 0.59 bc	49.23 ± 1.23 a
S4	49.29 ± 0.73 a	43.65 ± 0.81 a	49.95 ± 0.78 bc	54.33 ± 0.91 a	51.31 ± 1.13 bc	50.76 ± 0.47 a
S5	46.70 ± 1.25 a	44.61 ± 0.76 a	52.31 ± 1.25 ab	53.25 ± 0.85 a	52.08 ± 0.49 ab	51.61 ± 0.70 a
S6	49.01 ± 1.48 a	44.93 ± 1.17 a	51.66 ± 1.20 ab	53.23 ± 0.67 a	52.60 ± 1.31 ab	50.98 ± 0.98 a
S7	46.00 ± 2.69 a	44.16 ± 0.69 a	53.20 ± 1.07 a	55.13 ± 0.79 a	54.93 ± 1.48 a	52.81 ± 1.20 a
p value	0.066	0.472	0.000	0.255	0.000	0.076
Fertilization (F)						
No fertilization	47.44 ± 0.81 b	43.32 ± 0.30 b	49.21 ± 050 b	53.65 ± 0.25 a	49.62 ± 0.38 b	49.77 ± 0.40 b
Fertilization	48.87 ± 0.49 a	44.78 ± 0.39 a	52.31 ± 0.50 a	53.92 ± 0.44 a	52.72 ± 0.78 a	52.87 ± 0.42 a
p value	0.031	0.006	0.000	0.583	0.000	0.000

Note: Means and standard errors are reported. Within each column, values with different letters are significantly different at $p \leq 0.05$, according to Tukey's HSD test. S0, soil spraying with water only; S1, seed coating at a dose of 100 g q^{-1} of seeds; S2, soil spraying after sowing at a dose of 1 kg ha^{-1} of microbial complex; S3, soil spraying after sowing at a dose of 2 kg ha^{-1} of microbial complex; S4, soil spraying after sowing at a dose of 3 kg ha^{-1} of microbial complex; S5, S1 + S2; S6, S1 + S3; S7, S1 + S4.

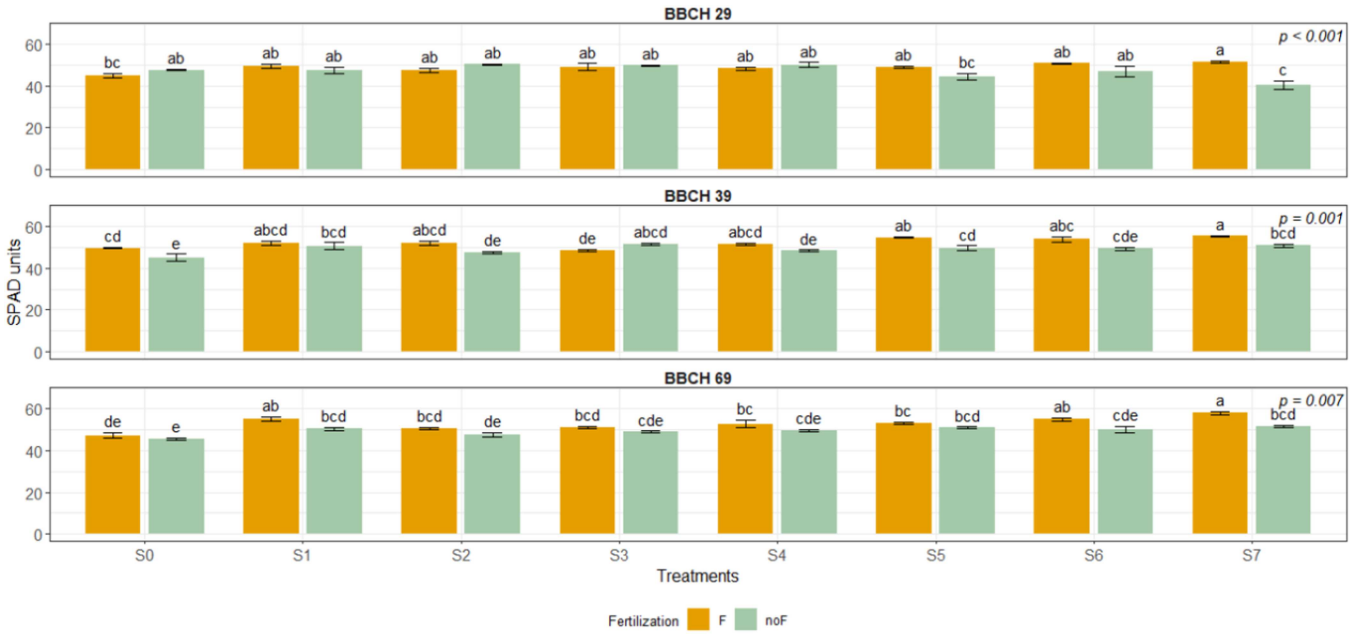


FIGURE 2 | Influence of the application × fertilization interaction on SPAD content at Sparacia experimental field. For each data series, values with different letters are significantly different at $p \leq 0.05$, according to Tukey's HSD test. Error bars represent standard error. F, fertilization; noF, unfertilized; S0, soil spraying with water only; S1, seed coating at a dose of 100 g q^{-1} of seeds; S2, soil spraying after sowing at a dose of 1 kg ha^{-1} of microbial complex; S3, soil spraying after sowing at a dose of 2 kg ha^{-1} of microbial complex; S4, soil spraying after sowing at a dose of 3 kg ha^{-1} of microbial complex; S5, S1 + S2; S6, S1 + S3; S7, S1 + S4.

at BBCH39 and BBCH69 (45.20 ± 1.65 and 45.73 ± 0.41, respectively) (Figure 2). In Carboj, only the fertilization treatment was significant across all three sampling dates, with no other effects from microbial application or the A×F interaction (Table S2). Also, for the physiological parameter, several microbial treatments improved SPAD compared to the unfertilized control (S0 × no fert), although the magnitude of this effect varied across phenological stages (Table 2). At Sparacia, intermediate microbial applications, such as S1 × noF, S3 × noF, S5 × noF and S6 × noF, showed higher chlorophyll levels than S0 × noF at BBCH39 and BBCH69, with increases generally ranging from +3 to +10 SPAD units, depending on the stage. Even under nutrient-limited conditions, these treatments maintained SPAD values close to or slightly above those

TABLE 3 | Stomatal conductance measured at two wheat growth stages (BBCH 29 and BBCH 39) at the two Sicilian sites (Sparacia and Campo Carboj) as affected by application treatment (A: S0–S7) and fertilization (F: no fertilization vs. fertilization).

	G_s (mmol m^{-2} s^{-1}) BBCH 29		G_s (mmol m^{-2} s^{-1}) BBCH 39	
Treatments	**Sparacia**	**Carboj**	**Sparacia**	**Carboj**
Application (A)				
S0	344.82 ± 9.43 c	349.30 ± 8.35 a	380.96 ± 29.0 c	244.04 ± 8.22 a
S1	449.30 ± 10.8 ab	353.23 ± 10.7 a	481.36 ± 17.3 ab	251.60 ± 12.9 a
S2	347.07 ± 7.77 c	339.06 ± a	423.83 ± 16.7 bc	232.73 ± 7.56 a
S3	425.00 ± 23.5 b	367.73 ± 14.4 a	481.46 ± 26.2 ab	234.56 ± 8.99 a
S4	452.92 ± 11.2 ab	348.70 ± 8.34 a	501.73 ± 24.9 a	263.56 ± 9.42 a
S5	467.76 ± 13.0 ab	346.16 ± 10.3 a	523.82 ± 21.3 a	247.70 ± 7.07 a
S6	470.06 ± 29.5 ab	357.03 ± 10.3 a	503.26 ± 24.3 a	247.60 ± 11.7 a
S7	483.28 ± 27.3 a	358.10 ± 10.1 a	507.56 ± 28.3 a	263.20 ± 10.9 a
p value	0.000	0.699	0.000	0.624
Fertilization (F)				
No fertilization	410.41 ± 10.8 b	354.65 ± 5.41 a	454.88 ± 5.89 b	249.77 ± 6.37 a
Fertilization	449.65 ± 14.7 a	350.17 ± 4.83 a	496.12 ± 18.5 a	246.48 ± 3.28 a
p value	0.000	0.556	0.000	0.209

Note: Means and standard errors are reported. Within each column, values with different letters are significantly different at $p \leq 0.05$, according to Tukey's HSD test. G_s, stomatal conductance; S0, soil spraying with water only; S1, seed coating at a dose of 100 g q^{-1} of seeds; S2, soil spraying after sowing at a dose of 1 kg ha^{-1} of microbial complex; S3, soil spraying after sowing at a dose of 2 kg ha^{-1} of microbial complex; S4, soil spraying after sowing at a dose of 3 kg ha^{-1} of microbial complex; S5, S1 + S2; S6, S1 + S3; S7, S1 + S4.

seen in several fertilized combinations, indicating that microbial inoculation supported chlorophyll retention and delayed senescence without mineral inputs. Specifically, at BBCH29, the variation among unfertilized microbial treatments was narrower; nonetheless, S2 × noF, S3 × noF, S4 × noF and S6 × noF all exhibited SPAD values equal to or higher than the unfertilized control, whereas S5 × noF and S7 × noF showed more pronounced reductions (Figure 2). The same was observed at Carboj, where SPAD values were more uniform across unfertilized treatments. However, microbial inoculation still tended to produce slightly higher SPAD values than the control, especially for S3 × noF, S6 × noF and S7 × noF, which consistently ranked among the highest in the unfertilized group, although these differences were not statistically significant (Table 2).

Regarding G_s, the Sparacia site showed highly statistical differences for the repeated measures ANOVA and for the two survey dates. No effect was detected at Carboj ($p > 0.05$) for either A, F or A×F across each assessment date (Table S5).

Regarding G_s, a significant effect of application was observed at Sparacia at both measurement times ($p < 0.001$), with S7 and S5 showing the highest values although not showing statistical differences with S6 and S4 and in the survey date 39 of the BBCH scale with S3. On both dates, G_s values were detected in S0 (Table 3).

Additionally, fertilization positively influenced G_s at Sparacia ($p < 0.001$), as did the A × F interaction, with S7 × F at BBCH29 (541.66 ± 8.3 mmol m^{-2} s^{-1}) and S5 × F at BBCH39 (568.77 ± 10.1 mmol m^{-2} s^{-1}), displaying the highest mean values, which were significantly higher than all other treatments. The lowest performance was recorded for the S0 × noF at both recording dates. Under unfertilized conditions at Sparacia, several microbial treatments improved G_s relative to the unfertilized control. At BBCH29, notable increases were observed in S5 × noF (+44%) and S4 × noF (+36%) compared to S0 × noF, with additional positive responses in S3 × noF and S7 × noF. A similar pattern emerged at BBCH 39, where S5×noF and S3×noF showed higher conductance than the unfertilized control (Figure 3).

Regarding NDVI, application methods, fertilization and the A×F interaction showed statistical differences both in the repeated measures ANOVA and during the different observation phases in the Sparacia experimental field. At the Carboj field, statistical differences were detected for all observation dates for application, while regarding fertilization, statistical differences were found only for repeated measurements and for observation dates 39 and 69 of the BBCH scale. In the latter environment, the A×F interaction was significant only for repeated measurements and during phase 69 of the BBCH scale (Table S6).

Table 4 presents the main effects of application timing and fertilization on vegetative vigour (NDVI) across the three sampling dates and the two experimental sites. Specifically, at both locations, the S6 and S7 applications exhibited the highest mean NDVI values, while the lowest values were recorded with the S0 application. Regarding fertilization, significant statistical differences were consistently observed at the Sparacia site; conversely, at Campo Carboj, statistical significance was only detected at the BBCH 39 and 69 growth stages. For this treatment (fertilization), the highest NDVI values were invariably obtained in the fertilized plots.

The A × F interaction was always significant for plants in Sparacia (Figure 4A), with S7 × F at BBCH 29 (0.53 ± 0.003;

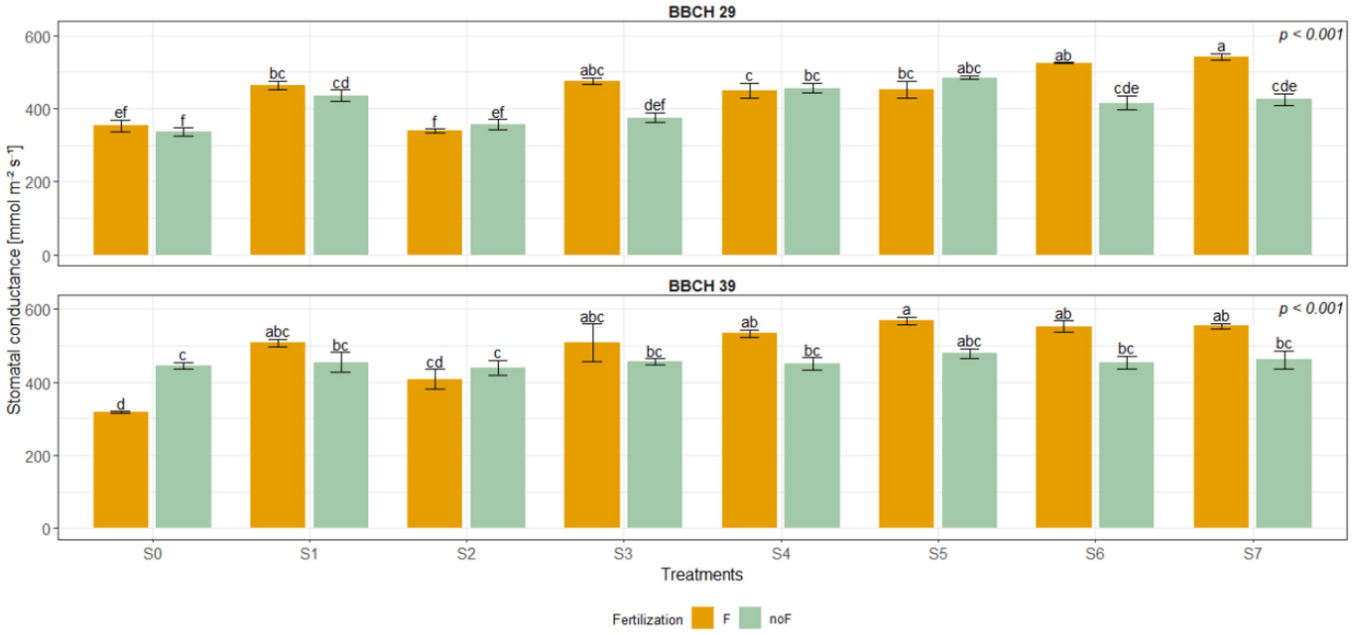


FIGURE 3 | Influence of the application × fertilization interaction on Stomatal conductance content at Sparacia experimental field. For each data series, values with different letters are significantly different at $p \leq 0.05$, according to Tukey's HSD test. Error bars represent standard error. F, fertilization; noF, unfertilized; S0, soil spraying with water only; S1, seed coating at a dose of 100 g q^{-1} of seeds; S2, soil spraying after sowing at a dose of 1 kg ha^{-1} of microbial complex; S3, soil spraying after sowing at a dose of 2 kg ha^{-1} of microbial complex; S4, soil spraying after sowing at a dose of 3 kg ha^{-1} of microbial complex; S5, S1 + S2; S6, S1 + S3; S7, S1 + S4.

TABLE 4 | NDVI measured at three wheat growth stages (BBCH 29, BBCH 39 and BBCH 69) at the two Sicilian sites (Sparacia and Campo Carboj) as affected by application treatment (A: S0–S7) and fertilization (F: no fertilization vs. fertilization).

Treatments	NDVI BBCH 29		NDVI BBCH 39		NDVI BBCH 69	
Application (A)	Sparacia	Carboj	Sparacia	Carboj	Sparacia	Carboj
S0	0.47 ± 0.01c	0.65 ± 0.04 d	0.78 ± 0.02 c	0.80 ± 0.01 b	0.49 ± 0.02 b	0.57 ± 0.01 b
S1	0.48 ± 0.01 bc	0.66 ± 0.02 d	0.81 ± 0.01 ab	0.81 ± 0.02 ab	0.55 ± 0.01 a	0.61 ± 0.02 ab
S2	0.47 ± 0.01 c	0.68 ± 0.04 c	0.78 ± 0.03 bc	0.83 ± 0.01 ab	0.56 ± 0.02 a	0.63 ± 0.01 ab
S3	0.47 ± 0.01 c	0.68 ± 0.01 c	0.81 ± 0.01 a	0.81 ± 0.01 ab	0.56 ± 0.04 a	0.65 ± 0.01 a
S4	0.49 ± 0.01 bc	0.72 ± 0.03 b	0.81 ± 0.01 a	0.82 ± 0.01 ab	057 ± 0.03 a	0.63 ± 0.01 ab
S5	0.50 ± 0.01 ab	0.72 ± 0.03 b	0.82 ± 0.01 a	0.83 ± 0.01 ab	058 ± 0.01 a	0.63 ± 0.01 ab
S6	0.51 ± 0.01 ab	0.78 ± 0.03 a	0.83 ± 0.01 a	0.84 ± 0.01 a	056 ± 0.01 a	0.65 ± 0.01 a
S7	0.52 ± 0.01 a	0.76 ± 0.02 ab	0.83 ± 0.01 a	0.83 ± 0.01 ab	0.59 ± 0.01 a	0.64 ± 0.01 a
p value	0.000	0.047	0.000	0.049	0.000	0.014
Fertilization (F)						
No fertilization	0.47 ± 0.01 b	0.70 ± 0.01 a	0.77 ± 0.01 b	0.80 ± 0.01 b	0.51 ± 0.01 b	0.60 ± 0.01 b
Fertilization	0.50 ± 0.01 a	0.72 ± 0.01 a	0.85 ± 0.01 a	0.84 ± 0.01 a	0.60 ± 0.01 a	0.65 ± 0.01 a
p value	0.000	0.310	0.000	0.000	0.000	0.000

Note: Means and standard errors are reported. Within each column, values with different letters are significantly different at $p \leq 0.05$, according to Tukey's HSD test. S0, soil spraying with water only; S1, seed coating at a dose of 100 g q^{-1} of seeds; S2, soil spraying after sowing at a dose of 1 kg ha^{-1} of microbial complex; S3, soil spraying after sowing at a dose of 2 kg ha^{-1} of microbial complex; S4, soil spraying after sowing at a dose of 3 kg ha^{-1} of microbial complex; S5, S1 + S2; S6, S1 + S3; S7, S1 + S4.

$p = 0.003$), S1 × F, S2 × F and S3 × F at BBCH39 (0.58 ± 0.012; $p < 0.001$) and S3 × F at BBCH69 (0.66 ± 0.006; $p < 0.001$). At Sparacia, under unfertilized conditions, several microbial treatments consistently showed higher NDVI values than the unfertilized control. Already at BBCH 29, treatments S5 × noF, S6 × noF and S7 × noF showed modest but clear increases in canopy reflectance. As the crop progressed to BBCH 39, most noF treatments showed enhanced canopy vigour compared with S0 × noF, with the exception of S2 × noF. By BBCH69, nearly all microbial treatments outperformed the control, suggesting that inoculation helped sustain a denser, more persistent canopy during later stages.

At Carboj (Figure 4B), the A × F interaction was significant only at BBCH69 ($p = 0.001$), where S3-4-5-6-7 × F outperformed the non-fertilized treatment but not the other fertilized

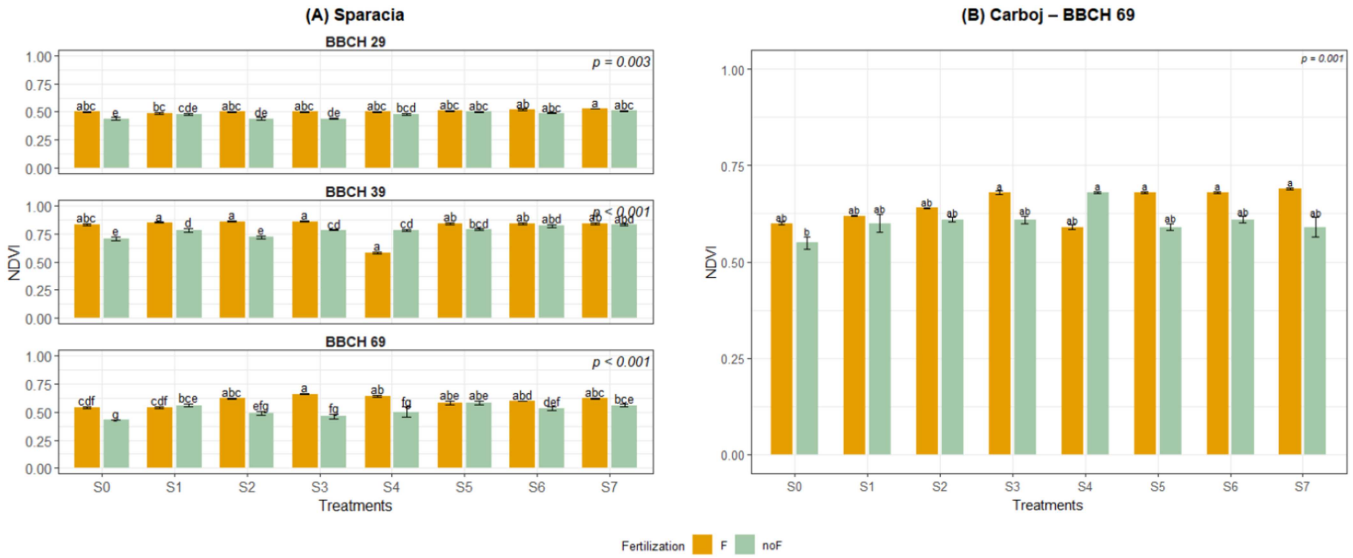


FIGURE 4 | Influence of the application × fertilization interaction on NDVI at Sparacia (A) and Campo Carboj (B) experimental field. For each data series, values with different letters are significantly different at $p \leq 0.05$, according to Tukey's HSD test. Error bars represent standard error. F, fertilization; noF, unfertilized; S0, soil spraying with water only; S1, seed coating at a dose of 100 g q^{-1} of seeds; S2, soil spraying after sowing at a dose of 1 kg ha^{-1} of microbial complex; S3, soil spraying after sowing at a dose of 2 kg ha^{-1} of microbial complex; S4, soil spraying after sowing at a dose of 3 kg ha^{-1} of microbial complex; S5, S1 + S2; S6, S1 + S3; S7, S1 + S4.

treatments (Figure 4B). Similar trends were observed under non-fertilized conditions at Carboj, though responses were smaller. The positive effect of microbial inoculation was most evident at BBCH69, with treatments such as S3 × noF, S6 × noF and S7 × noF exceeding the unfertilized control by approximately +0.04 to +0.06 NDVI units.

4 | Discussion

Across both experimental sites, the microbial consortium significantly enhanced soil P_2O_5 availability, confirming the ability of *Bacillus* spp. to mobilize P, as documented by a substantial body of research through mechanisms such as organic acid secretion (De Oliveira-Paiva et al. 2024; Iqbal et al. 2024; Pang et al. 2024), phosphatase enzyme activity (Luo et al. 2024; Xiaoxia et al. 2021) and pH modulation in the rhizosphere (Nagah et al. 2024; Saeid et al. 2018), which collectively mobilize otherwise inaccessible soil P. It's not surprising then that the most evident effects were observed at Sparacia, where soils were more calcareous and clay-rich—conditions under which P is strongly immobilized (Brownrigg et al. 2022; von Wandruszka 2006). Here, several microbial treatments (especially S1, S5, S6, S7) produced P increases comparable to those observed with fertilizer application and, in some cases, exceeding +50% over the unfertilized control. As recently highlighted by Mohanty et al. (2024), the effectiveness of phosphate-solubilizing bacteria (PSB) in such alkaline and calcareous environments is often limited not by their intrinsic solubilization potential but by their ability to survive and establish in the rhizosphere. The significant P increases achieved by strategies S6 and S7 at Sparacia suggest that a multi-modal inoculation (seed + soil) provides a 'buffer effect' that facilitates microbial colonization, allowing the consortium to effectively bypass the geochemical P-fixation typical of Mediterranean clay soils. These results are consistent with very recent studies carried out on maize shoot P accumulation (De Andrade Rodrigues Severo et al. 2023), also on wheat productivity (Elhaissoufi et al. 2024) and on general field application as a fertilization practice capable of replacing the traditional fertilization practices (Aslam et al. 2024; Długosz and Piotrowska-Długosz 2023; Enriquez-León et al. 2025). What's also interesting and worth noticing for the implications that might follow is that the improvements observed even in unfertilized plots (e.g., S6 × noF and S7 × noF) indicate that the consortium could mobilize native soil P, partially compensating for the absence of mineral fertilization. This response aligns with previous findings showing that multi-strain *Bacillus* formulations are particularly effective in P-fixing Mediterranean soils, where biological solubilization can significantly increase P availability (Timofeeva et al. 2023; Iqbal et al. 2024). Although the response pattern at Carboj was similar but less pronounced, this might be due to more favourable baseline soil fertility and texture (Tremblay et al. 2012; L. Wang et al. 2022), which reduces the relative contribution of microbial P-solubilization. This highlights the need for site-specific microbial management, moving away from a 'one-size-fits-all' biostimulant application toward a more tailored strategy based on the soil's physical–chemical matrix. Nonetheless, the microbial treatments still improved P availability relative to the control, supporting the consortium's resilience across contrasting environments.

Microbial application significantly influenced plant height at both sites, with treatment S7 consistently outperforming the control. The positive effect was especially evident at Sparacia, where non-fertilized microbial treatments increased height by up to +23% compared to the unfertilized control. This suggests that the microbial consortium supported shoot growth not only by improving nutrient availability but also by stimulating phytohormone-mediated growth, as widely reported for *Bacillus* spp. (e.g., auxin and cytokinin production) (Kudoyarova et al. 2019; Ortíz-Castro et al. 2008). Also, for plant height, the narrower response observed at Carboj reflects more favourable edaphic conditions and lower overall growth constraints. Yet, the consistent advantage of microbial treatments under both fertilized and unfertilized conditions

highlights the broad efficacy of the consortium (Santoyo et al. 2021). The tiller number and fertile spikelets showed only minor numerical variations among the unfertilized microbial treatments and the unfertilized control, confirming in wheat the limited sensitivity of these reproductive traits to microbial application under no-input conditions (Bicego et al. 2024; Kaur et al. 2024). This lack of response suggests that early phenological development was predominantly governed by environmental factors rather than biological inputs. Specifically, the low temperatures recorded during the tillering phase (January–February), which frequently dropped below 0°C at the Sparacia site (Figure 1), likely exerted a primary limiting effect on tiller emergence and subsequent spike differentiation, potentially masking any growth-promoting influence of the microbial inoculants. This aligns with the 'context-dependency' of microbial efficacy, where extreme thermal constraints can define the upper limit of plant plastic response, regardless of the biostimulant's potential (Mohanty et al. 2024). Overall, these first outcomes indicate that the vegetative component of plant development was more responsive to microbial inoculation than the reproductive components, particularly in the clay-rich environment of Sparacia, consistent with other studies on wheat and other cereal crops (El-Sorady et al. 2022; Hett et al. 2023).

The microbial consortium strongly influenced physiological performance, particularly at Sparacia. SPAD values demonstrated that microbial application sustained higher chlorophyll content, especially in fertilized plots, but also under nutrient-limited conditions. Treatments such as S1×noF, S3×noF and S6×noF consistently exceeded the unfertilized control, suggesting improved nitrogen assimilation and delayed senescence. These findings are consistent with reported increases in N uptake and chlorophyll stability following inoculation with *Bacillus* PGPR in many species. In *Arabidopsis*, *Bacillus* PGPR mixtures significantly increased chlorophyll content and upregulated genes involved in nitrate and ammonium uptake, thereby enhancing nutrient accumulation and plant growth (Calvo et al. 2019). Similarly, *Bacillus velezensis* inoculation in strawberries increased chlorophyll content by 2.7%–6.8%, improving mineral nutrition and yield (Chebotar et al. 2022). Under drought stress, *Bacillus* strains elevated chlorophyll levels and improved physiological resilience in wheat, contributing to better growth (Salem et al. 2024). *Bacillus* inoculation also reduced nitrate accumulation while increasing ammonium uptake and chlorophyll index in hydroponic arugula, indicating improved nitrogen assimilation and photosynthetic capacity (da Silva Oliveira et al. 2023). Although SPAD, NDVI and G_s are indirect proxies of nutrient status, their consistent improvement in response to *Bacillus* inoculation—coupled with the observed increase in soil Olsen P and plant height—provides a coherent indication of enhanced P-acquisition. In durum wheat, improved P-nutrition is known to sustain photosynthetic efficiency and cell elongation, which in our study resulted in taller plants and more vigorous canopies even under unfertilized conditions. While direct P-uptake analysis was not performed in this first part of the study, these physiological and morphological 'feedbacks' clearly reflect the plant's positive response to increased P-availability mediated by the microbial consortium.

G_s data revealed a similar pattern. The higher overall G_s recorded at Sparacia compared to Carboj can be attributed to the significantly greater cumulative rainfall and intensity at this site. As shown in Figure 1, Sparacia received nearly double the total precipitation (approx. 480 mm) compared to Carboj (approx. 210 mm) during the study period. This higher water input, likely coupled with the greater water-holding capacity of the clay-rich soil at Sparacia, maintained a more favourable leaf water potential. At this site, microbial treatments enhanced stomatal activity, with S5 × noF and S4 × noF showing substantial increases even without fertilization. Higher conductance likely reflected improved root function and water relations, possibly linked to microbial modulation of stress hormones such as ethylene (Ravanbakhsh et al. 2018). In fact, it's recognized that many PGPR produce the enzyme ACC deaminase, which lowers plant ethylene levels by breaking down its precursor ACC, thereby reducing ethylene-induced growth inhibition and enhancing root growth and stress tolerance (Brígido et al. 2015). These environmental factors, indirectly reflected in our G_s and SPAD data, confirm that the success of Bacillus inoculation is strictly context-dependent, providing better support when environmental constraints (such as soil texture or moisture fluctuations) are more pronounced. Additionally, microbes have been shown to play a key role in altering ethylene biosynthesis genes in plants, leading to improved nutrient uptake and reduced stress damage, as seen in rice exposed to heavy metals (Qian et al. 2022). These environmental factors, indirectly reflected in our G_s and SPAD data, confirm that the success of Bacillus inoculation is strictly context-dependent, providing better support when environmental constraints (such as soil texture or moisture fluctuations) are more pronounced. The lack of response at Carboj may indicate better water availability and soil aeration, reducing the microbial influence (Carter et al. 2023; Ulrich et al. 2019). Many studies show that microbial symbionts can boost G_s by improving leaf water potential and modifying stomatal morphology under stress. However, these effects are more evident under water or salinity stress (Ortiz et al. 2015).

NDVI measurements further confirmed the beneficial impact of microbial inoculation. Vegetation vigour increased consistently with microbial intensity, with S6 and S7 outperforming all other treatments throughout the season. Notably, under unfertilized conditions at Sparacia, microbial treatments improved canopy greenness from early tillering (BBCH29) to grain filling (BBCH69), indicating sustained physiological activity and increased photosynthetic capacity, with clear and positive implications also on grain yield and quality of production, as reported on buckwheat and tomato (Witkowicz et al. 2021; Quintarelli et al. 2024). At Carboj, the effects were more minor yet detectable, particularly at BBCH69, suggesting that microbial inoculation supported canopy maintenance even under mild environmental constraints (Assainar et al. 2018). Interestingly, despite the lower plant height recorded at Carboj compared to Sparacia, the former site exhibited generally higher absolute NDVI values. This discrepancy is clearly explained by the different tillering capacity observed between the two locations: at Carboj, the number of tillers was significantly higher (ranging from 8.73 to 10.36) compared to Sparacia (3.37 to 4.13). This higher tillering density at the sandy site resulted in a more continuous and dense canopy cover, which increased the spectral reflectance (NDVI). Conversely, at the clayey site (Sparacia), the taller but more sparse plants (fewer tillers) led to a more fragmented canopy with more visible bare soil, explaining the lower NDVI values despite the greater vertical growth.

The observed increases in soil P_2O_5—especially in the more P-constraining soil—and the consistent improvements in plant

height and canopy/physiological indicators even under unfertilized conditions support the premise that microbial consortia can partially mobilize native soil P and sustain crop performance, with combined seed + soil approaches emerging as particularly effective for field establishment and functional expression (Aslam et al. 2024; Iqbal et al. 2024; Pang et al. 2024). By integrating seed coating with soil drenching (S6, S7), we addressed one of the major bottlenecks in microbial application: the inconsistency of establishment (Mohanty et al. 2024). Our findings suggest that this multi-modal inoculation approach is essential for field success, offering a robust mechanistic framework for the development of sustainable fertilization programs in Mediterranean durum wheat systems.

5 | Conclusions

A key outcome of this study is the clear benefit of microbial treatments under unfertilized conditions, where several inoculation strategies—particularly at Sparacia—enhanced soil P availability, plant height, SPAD, Gs and NDVI compared with the unfertilized control. These results confirm the capacity of the multi-strain *Bacillus* consortium to partially compensate for the absence of mineral fertilizers and reinforce its potential as a component of integrated nutrient management aimed at reducing chemical inputs. The strong performance of combined seed and soil applications (S6, S7) further underscores the value of multi-modal inoculation approaches that improve microbial establishment and functional expression in the field.

Despite these promising results, a limitation of the current study is the lack of a detailed characterization of the native soil microbiota at the experimental sites. The interaction between the applied *Bacillus* consortium and the resident microbial communities is a critical factor, as native biology can either diminish the inoculants' efficacy through competition or enhance it through synergistic effects. Understanding these dynamics is essential, as the long-term persistence of introduced strains and their integration into the soil ecosystem will significantly influence the overall productivity and resilience of agroecosystems.

To provide a complete agronomic assessment of the proposed strategies, the research was structured into two complementary phases. Since the present study (Part I) focused primarily on soil dynamics and plant physiological responses during the vegetative stages, a forthcoming companion paper (Part II) will provide a comprehensive analysis of yield components, total grain production and grain quality traits (e.g., protein content, gluten strength, yellow index and test weight), as well as their technological implications for the milling and pasta industries. This sequential approach is essential to determine whether the physiological enhancements observed here translate into measurable improvements at harvest and to quantify the full agronomic value of microbial inoculants.

Furthermore, the observed differences between experimental environments highlight that the context of application is critical: selecting the most responsive site can maximize the benefits of microbial treatments. Additional investigations should also examine the long-term persistence of inoculant strains, their interactions with native soil microbiota and the mechanistic pathways underlying nutrient mobilization and plant growth promotion. Finally, optimizing application strategies and assessing performance under climate-related stresses, combined with economic and environmental analyses, will support the development of reliable and sustainable microbial fertilization programs.

Author Contributions

Nicolò Iacuzzi and Teresa Tuttolomondo: conceptualization. Nicolò Iacuzzi, Noemi Tortorici and Mauro Sarno: methodology. Antonio Giovino and Giuseppe Indovino: software. Nicolò Iacuzzi, Teresa Tuttolomondo and Mauro Sarno: validation. Nicolò Iacuzzi, Noemi Tortorici, Antonio Giovino and Teresa Tuttolomondo: formal analysis. Noemi Tortorici, Giuseppe Indovino and Nicolò Iacuzzi: investigation. Teresa Tuttolomondo and Nicolò Iacuzzi: resources. Nicolò Iacuzzi and Noemi Tortorici: data curation. Nicolò Iacuzzi, Roberto Marceddu and Noemi Tortorici: writing–original draft preparation. Nicolò Iacuzzi, Antonio Giovino, Teresa Tuttolomondo and Mauro Sarno: writing–review and editing. Teresa Tuttolomondo, Noemi Tortorici and Antonio Giovino: visualization. Teresa Tuttolomondo and Nicolò Iacuzzi: supervision. Nicolò Iacuzzi and Teresa Tuttolomondo: project administration. Nicolò Iacuzzi: funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting File 1: sae270179-sup-0001-Figure_S1.docx.
Supporting File 2: sae270179-sup-0002-Figure_S2.docx.
Supporting File 3: sae270179-sup-0003-Table_S1.docx.
Supporting File 4: sae270179-sup-0004-Table_S2.docx.
Supporting File 5: sae270179-sup-0005-Table_S3.docx.
Supporting File 6: sae270179-sup-0006-Table_S4.docx.
Supporting File 7: sae270179-sup-0007-Table_S5.docx.
Supporting File 8: sae270179-sup-0008-Table_S6.docx.