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Divergent effects of a *Bacillus* biofertilizer immobilized in guinea pig manure biochar: Boosting productivity vs. enhancing nutritional quality in maca monocultures

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ABSTRACT

Intensive monoculture of maca (*Lepidium meyenii*) in the Peruvian Andes induces edaphic degradation, requiring sustainable amendments. We evaluated the agronomic and nutritional effects of a *Bacillus subtilis* biofertilizer immobilized in guinea-pig manure biochar, contrasting it with its individual components. A field experiment was conducted in Junín, Peru (4449 m a.s.l.) using a randomized complete block design with four treatments: control, biochar alone (3 L m^{-2}), liquid *B. subtilis* (125 L ha^{-1}, 10^7 CFU mL^{-1}) and biochar + *B. subtilis*. Plant establishment, yield, nutritional quality, and hypocotyl nutrient content were evaluated. Results revealed a functional divergence between treatments. Biochar-based treatments (alone and combined) significantly increased plant density (up to 49 vs. 29 m^{-2} in the control) and yield (1290–1500 vs. 550 kg ha^{-1}), associated with greater K accumulation (up to 22,252 mg kg^{-1}; $p < 0.001$). In contrast, *B. subtilis* alone primarily enhanced nutritional quality, resulting in the highest protein (3.88 g 100 g^{-1}) and fat (0.24 g 100 g^{-1}) contents. The combined treatment achieved the highest individual plant weight (10.1 g) and high yields; however, it did not retain the quality-related benefits, exhibiting the lowest protein content (3.34 g 100 g^{-1}). Redundancy analysis (RDA) confirmed this functional separation: biochar was associated with a mineral–productivity gradient, whereas *B. subtilis* aligned with a proximate–nutritional gradient. These findings indicate that biochar primarily acts as edaphic conditioner driving productivity, while *B. subtilis* enhances nutritional quality. The absence of full synergism in the combined treatment suggests independent modes of action, resulting in a yield–quality trade-off. This enables the design of differentiated fertilization strategies tailored either toward maximum productivity (biochar alone) or premium nutritional quality (*B. subtilis* alone).

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1. Introduction

Maca (*L. meyenii Walp.*) is an emblematic crop of central Andes, adapted to high-altitude environments where low temperatures, intense solar radiation, and limited soil resources constrain plant physiology and productivity (Huarancca Reyes et al., 2020). In these conditions, its value as a functional food and nutraceutical raw material has positioned it within expanding commercial chains, generating direct economic benefits for high-Andean rural communities (Wasilewicz and Grienke, 2026). This scenario has promoted the expansion of cultivated areas and management intensification, often under monocropping systems with high soil-use pressure (Solórzano-Acosta and Quispe, 2024). Although monoculture may respond to market incentives, in fragile high-altitude systems it increases agroecosystem vulnerability if not accompanied by appropriate soil fertility management and biological conservation strategies (Huarancca Reyes et al., 2020; Solórzano-Acosta and Quispe, 2024).

Continuous maca monocropping has been consistently associated with signs of edaphic degradation, including reduced organic matter content, soil acidification, nutrient imbalances, and loss of biological functionality—issues recurrently reported in high-Andean production zones (Solórzano-Acosta and Quispe, 2024). At the microbial level, management intensification alters the structure and functional potential of soil communities, with direct implications for nutrient cycling and system stability (Custodio et al., 2021). Complementary assessments conducted in high-Andean soils disturbed by maca cultivation have documented shifts in bacterial diversity across the crop cycle and along gradients of land-use intensity, suggesting a trajectory of functional impoverishment when cultivation is repeated without adequate soil recovery (Huaraca-Meza et al., 2022). This progressive soil deterioration not only threatens the environmental sustainability of high-Andean agroecosystems but also compromises the productive and economic foundation of regions that depend on this emblematic crop.

In response to this challenge, contemporary agronomic research is oriented toward the development and validation of sustainable agricultural inputs. In this regard, biofertilizers based on plant growth-promoting bacteria (PGPB) are consolidating as a strategic alternative to restore soil processes and reduce dependence on chemical inputs, particularly in vulnerable production systems (Maciel-Rodríguez et al., 2025). PGPB play a pivotal role in the recovery of degraded soils and the enhancement of crop productivity through both direct mechanisms—such as nutrient mobilization and phytohormone production—and indirect mechanisms, including biocontrol and stress tolerance, thereby exerting beneficial effects within the rhizospheric microenvironment (de Andrade et al., 2023; Yang et al., 2024). Among PGPB, the genus *Bacillus* is particularly prominent due to its capacity to form endospores, a trait that provides advantages for storage stability and formulation, as well as its ability to colonize plant roots and produce metabolites with growth-promoting and protective functions (Patel et al., 2023; Yang et al., 2024). Strains of *Bacillus*, together with other PGPB genera reported in the high-Andean rhizosphere of maca, exhibit adaptations to cold and high-altitude environments, expressing relevant plant growth-promoting (PGP) traits such as stress tolerance and nutrient mobilization potential (Zúñiga et al., 2010; Ortiz-Ojeda et al., 2017).

However, microbial inoculants present a major constraint, namely their inconsistent performance under field conditions. Following application, microbial cells are exposed to multiple environmental stresses, including water deficit, temperature fluctuations, radiation, competition with native soil microbiota, and limited carbon availability. These factors reduce survival rates and root colonization, ultimately diminishing the expected agronomic effect (Saad et al., 2020). Therefore, beyond strain selection, it is essential to design effective delivery technologies, such as carriers, coatings, or encapsulation systems, that protect the microorganism and position it near the root zone, thereby increasing the likelihood of establishment (Bashan et al., 2014; Rocha et al., 2019). In this regard, the development of next-generation biofertilizers requires the integration of microbiology and materials science to ensure that the delivery matrix sustains viable populations and that the system remains functional under the environmental fluctuations typical of high-Andean agroecosystems.

One of the delivery technologies that has received considerable attention is biochar, due to its potential as a soil amendment to improve physical, chemical, and biological properties (Biederman and Harpole, 2013). Biochar can modify microbial habitats by providing surface area and porosity, altering soil pH and water retention capacity, and modulating nutrient cycling processes (Lehmann et al., 2011). In parallel, increasing interest has emerged in biochars derived from nutrient-rich residues, such as animal manures, which typically exhibit higher mineral and ash contents, with important implications for soil fertility (Rathnayake et al., 2023; Cui et al., 2025). Among mineral-rich organic residues, guinea pig manure (*Cavia porcellus*) stands out due to its nutrient contribution and its capacity to improve soil physical properties, including water retention and aggregate stability, thereby offering potential benefits for crop performance (Espejo Huerta et al., 2021; Calero-Rios et al., 2025). Moreover, its conversion into biochar represents a locally viable circular economy strategy, generating a material with elevated mineral content and properties that may contribute to the rehabilitation of acidic soils; however, its effectiveness must be validated for each crop and specific management condition (Ccopi et al., 2025; Solórzano, 2026). Within this framework, the immobilization of beneficial bacteria within porous biochar matrices emerges as an integrative strategy in which biochar functions as a protective microhabitat and reservoir, while the microorganism provides PGP functions that may be expressed with greater persistence in the rhizosphere. Nevertheless, the functional interaction between both components—and whether their effects are synergistic, additive, or even divergent—remains insufficiently explored under field conditions, particularly in maca monoculture systems.

In this context, the objective of the present study was to evaluate the agronomic performance of a biofertilizer based on *B. subtilis* immobilized in guinea pig manure–derived biochar under maca monoculture systems. Based on previous evidence highlighting the differentiated roles of biochar—as a soil conditioner and nutrient source—and *Bacillus* sp. as promoters of nutritional quality, we hypothesized that these inputs would exert functionally divergent effects: biochar would primarily enhance crop productivity, whereas *B. subtilis* would improve the nutritional quality of the hypocotyl. We further anticipated that the immobilized formulation would partially integrate both benefits.

2. Materials and methods

2.1. Study area

The field experiment was conducted during the 2024–2025 growing season in Chupaca, Junín district and province, Junín region, Peru (UTM WGS84 zone 18S: 398217 E, 8765007 S), at an altitude of 4449 m a. s. l. (Fig. 1). This area, located within the natural Puna region, forms part of the center of origin of *L. meyenii* (maca) and is characterized by an alpine tundra climate (ET, according to the Köppen classification) (Beck et al., 2018). During the experimental period, temperatures ranged from −3.8 to 16.8 °C in the rainy season and from −11.8 to 16.4 °C in the dry season, with a mean relative humidity of 79.6% and a total annual precipitation of 821.3 mm (data from the Junín meteorological station, SENAMHI, 2025).

Soil characterization was conducted at the Soil, Water and Foliar Analysis Laboratory (LABSAF) of the National Institute for Agrarian Innovation (INIA). The basic physicochemical properties of the experimental soil were as follows: sandy loam texture; moderately acidic reaction (pH 5.1; EPA Method 9045D) (U.S. EPA, 2004); 82.4 g kg^{-1} of organic matter (ISO 10694:1995), 4.21 mg kg^{-1} of nitrogen (ISO 13878:1998) determined by dry combustion; 128.27 mg kg^{-1} of

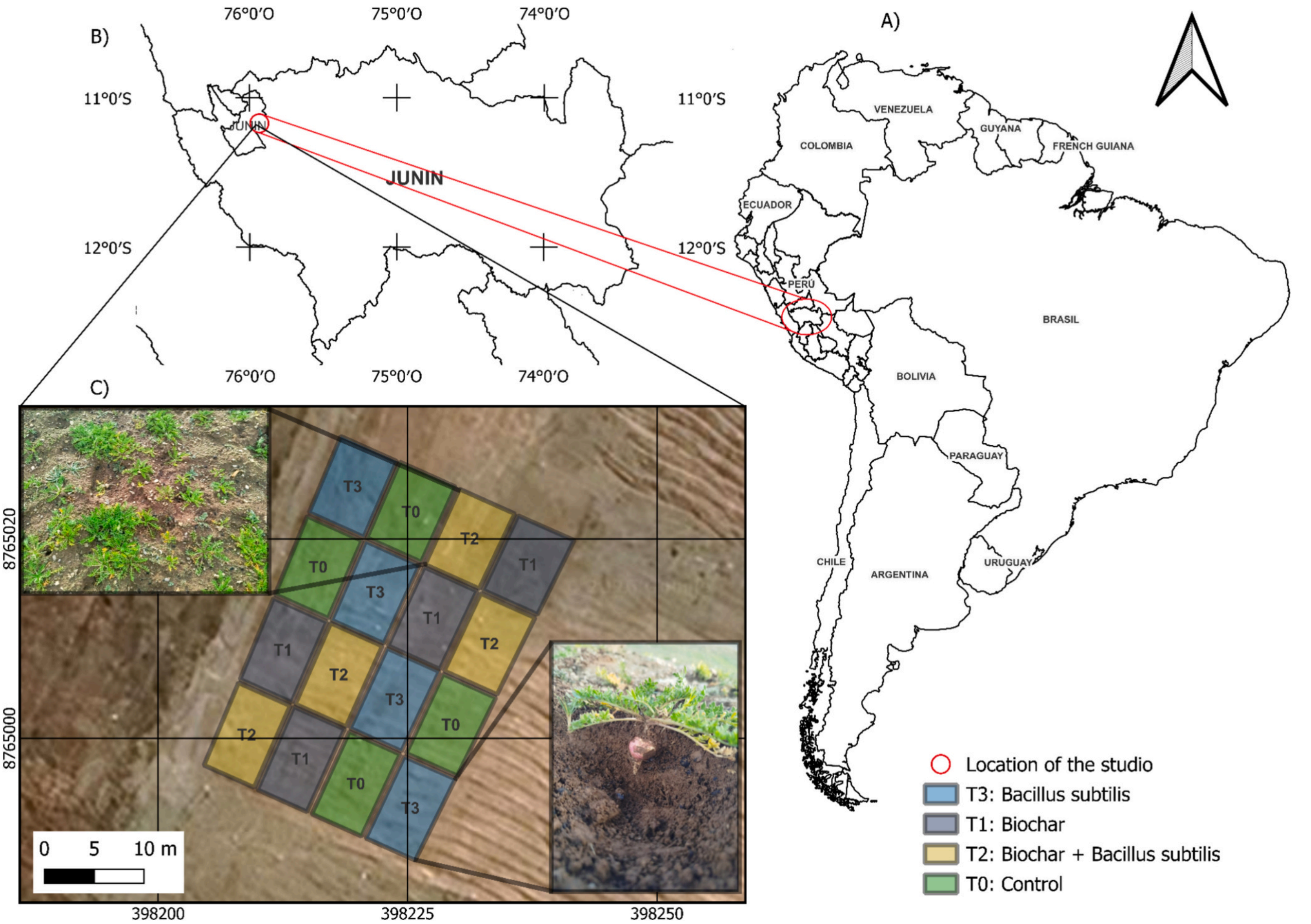

Fig. 1. Geographic location of the experimental area in the district of Junín, Peru, and schematic representation of the randomized complete block design (RCBD) implemented in the maca field trial.

available phosphorus (Bray and Kurtz; NOM-021-RECNAT-2000) (Semarnat, 2002); and 111.33 mg kg^{-1} of exchangeable potassium (1 N ammonium acetate extraction followed by MP-AES determination) (Balaram, 2020). Regarding phosphorus content, additional analyses of adjacent soils showed values ranging from 59 to 351 mg kg^{-1} (unpublished data), indicating that the area naturally exhibits high phosphorus availability, possibly associated with historical management practices or specific edaphic conditions of the region.

To evaluate the effects of the treatments on soil chemical properties, composite soil samples (0–20 cm depth) were collected from each experimental plot at harvest. Soil pH, electrical conductivity (EC), and exchangeable potassium (K) were determined using the same analytical procedures described for the initial soil characterization.

The experimental field had been cultivated continuously with maca (*Lepidium meyenii Walp.*) for five consecutive years prior to the establishment of the experiment. This production system is representative of traditional high-Andean maca cultivation, where organic management practices predominate and synthetic fertilizers are generally avoided to maintain product quality and market value. Before each growing season, the soil was conventionally tilled for seedbed preparation; however, no crop rotation was practiced, resulting in a continuous monoculture system. Consequently, soil fertility depended primarily on native nutrient reserves and natural nutrient cycling processes. No synthetic fertilizers, pesticides, or commercial biofertilizers had been applied during the previous cropping cycles.

2.2. Production process and physicochemical characterization of guinea pig manure–derived biochar

Biochar was produced from guinea pig manure collected from local farms in the province of Junín. The feedstock was sieved (5 mm mesh) to ensure homogeneity and air-dried for 3 days until reaching a residual moisture content <20%. Thermochemical conversion was carried out through slow pyrolysis in a fixed-bed reactor under a nitrogen atmosphere (2 L min^{-1} flow rate), with a heating rate of 10 °C min^{-1} until reaching a peak temperature of 450 °C, which was maintained for 3 h (residence time). The reactor was subsequently allowed to cool passively to room temperature for approximately 2 h. The process followed the protocols described by Yang et al. (2016) and Lu et al. (2020), with operational parameters within the recommended ranges for manure-derived biochars (Yu et al., 2019; Ayito et al., 2023; Mujtaba et al., 2021).

Biochar yield (calculated as final dry weight / initial dry weight × 100) was 32% (mean of three batches; range 30–35%). Physicochemical analysis of the resulting material revealed an alkaline pH of 8.4 (1:10 biochar: water ratio) and an electrical conductivity of 6.29 dS m^{-1} (1:10 aqueous extract), exceeding the 4 dS m^{-1} threshold for saline materials according to USDA classification criteria. The biochar exhibited an organic matter content of 40.4%, determined by loss-on-ignition at 550 °C, and high macronutrient concentrations, including 21,910 mg kg^{-1} of total phosphorus and 31,035 mg kg^{-1} of total potassium (quantified after aqua regia digestion and MP-AES determination; Balaram, 2020). The final product was stored in sealed polypropylene bags at room temperature (20–25 °C) and relative humidity <40% until field application.

2.3. Preparation of bioinputs according to the experimental treatments

The *B. subtilis* BACF strain (Genbank accession number MT982637), provided by the INIA Germplasm Bank, was used due to its documented plant growth-promoting (PGPR) traits (Solórzano-Acosta and Quispe, 2024). This strain is associated with increased root and total biomass, as well as leaf area and enhanced nutrient uptake efficiency in other crops, even though it was not identified as a phosphate solubilizer or a siderophore producer (Solórzano-Acosta and Quispe, 2024). For inoculum preparation, the microorganism was cultured in nutrient broth (0.5% peptone, 0.3% yeast extract, and 0.5% NaCl) adjusted to pH 6.8 and incubated for 72 h at 28 °C under constant agitation (150 rpm), reaching a cell density of 10^9 CFU mL^{-1}. Serial decimal dilutions were subsequently prepared from the mother culture using non-chlorinated water to obtain a final suspension of 10^7 CFU mL^{-1}, which was used as the field inoculum (Huasasquiche et al., 2024). The final concentration was verified by plate counting prior to field application.

For *B. subtilis* liquid treatment (T3), suspension of 10^7 CFU mL^{-1} was directly applied on the soil at a rate of 125 L ha^{-1}, reaching a final dosage of 1.25×10^{12} CFU ha^{-1}. The selected concentration is consistent with previous studies that have used 10^7–10^8 CFU mL^{-1} for plant growth promotion and biocontrol (Zapata-Narváez et al., 2024; Oyoque-Salcedo et al., 2025). Moreover, studies evaluating a wide range of inoculum concentrations (10^4–10^{10} CFU mL^{-1}) indicate that effective plant responses are achieved at intermediate levels, with no consistent additional benefit at higher concentrations (Bueno et al., 2022; Oyoque-Salcedo et al., 2025).

For the combined treatment (T2, biochar + *B. subtilis*), the inoculum was immobilized in guinea pig manure–derived biochar following an adapted protocol from Vlajkov et al. (2023). Briefly, 143.4 kg of guinea pig manure-derived biochar (bulk density = 239 kg m^{-3}; equivalent to 600 L), air-dried and sieved to 5 mm, were mixed with 7.5 L of the bacterial suspension (10^7 CFU mL^{-1}), 1.3 L of sugarcane molasses (used as a carbon source to facilitate microbial establishment), and 10 L of non-chlorinated water. The mixture was manually homogenized and incubated for 24 h at ambient temperature (7.3 ± 0.86 °C) under dark conditions to allow spore adsorption and initial colonization within the porous biochar matrix. After this period, the immobilized material was field-applied at a rate of 35.85 kg $plot^{-1}$, corresponding to 7.17 t ha^{-1} (equivalent to 150 L $plot^{-1}$ based on the measured bulk density).

2.4. Experimental design and treatments

The experiment was established under a randomized complete block design (RCBD) with four blocks and four treatments, resulting in a total of 16 experimental units (Fig. 1). Each experimental plot measured 10 × 5 m (50 m^2), and was separated by 1 m-wide buffer strips to minimize interference among treatments. Additionally, tillage operations were conducted perpendicular to the slope to prevent the runoff and displacement of applied inputs between adjacent plots.

The applied treatments were as follows: T0 (Control), consisting of traditional management without the application of soil amendments or synthetic fertilizers. T1 (Biochar), involving the application of air-dried guinea pig manure–derived biochar at a rate of 7.17 t ha^{-1} (0.717 kg m^{-2}; 35.85 kg $plot^{-1}$). The equivalent volumetric rate was 3 L m^{-2} based on a measured bulk density of 239 kg m^{-3}. T2 (Biochar + *B. subtilis*): corresponding to the application of the immobilized biofertilizer prepared as described in Section 2.3. For this treatment, a mixture composed of 143.4 kg of guinea pig manure-derived biochar (equivalent to 600 L; bulk density = 239 kg m^{-3}), 7.5 L of bacterial inoculum (10^7 CFU mL^{-1}), 1.3 L of molasses, and 10 L of water was prepared. The liquid phase (inoculum, molasses, and water) was completely absorbed into the porous biochar matrix, which functioned as a carrier. From this final mixture, 35.85 kg of biochar-containing material per plot were applied, providing a biochar application rate equivalent to 7.17 t ha^{-1} on a dry biochar basis, identical to that used in T1. The corresponding volumetric application rate was 150 L $plot^{-1}$. T3 (*B. subtilis* liquid); consisting of the application of 2.5 L of concentrated culture (10^9 CFU mL^{-1}) diluted in 10 L of non-chlorinated water per plot, corresponding to a field rate of 125 L ha^{-1} of the final suspension (approximately 1.25 × 10^{12} CFU ha^{-1}). The suspension was uniformly applied to the soil by manual spraying immediately after sowing.

2.5. Crop management

Sowing of *L. meyenii* was carried out during the 2024–2025 agricultural season, with planting conducted in November, following the traditional phenological calendar of the Junín region. The crop was grown under rainfed conditions, relying exclusively on seasonal precipitation (821 mm throughout the crop cycle). Broadcasting was used as the sowing method to ensure uniform seed distribution over the previously prepared soil surface. The seeding rate was 4 kg ha^{-1}, adjusted according to local production standards to achieve an optimal plant population at altitudes above 4400 m a.s.l. No synthetic fertilizers, pesticides, or additional chemical amendments were applied throughout the crop cycle in order to strictly evaluate the effects of the organic and microbiological biofertilization treatments on yield and nutritional quality. Crop management practices were limited to manual weed control and periodic phytosanitary monitoring, allowing hypocotyl development to be influenced solely by natural edaphoclimatic conditions and the applied experimental treatments. Harvesting was performed manually at physiological maturity, followed by immediate weighing and sampling for subsequent laboratory analyses.

2.6. Parameters evaluated

2.6.1. Biometric parameters and agronomic yield

Growth parameters were evaluated at the end of the crop phenological cycle, coinciding with harvest (July 2025). For plant population density determination, all plants within four 1 m^2 quadrats located in the central area of each plot were directly counted, excluding border areas. Leaf size was determined by measuring the length (from the base of the petiole to the leaf apex) of the longest leaf from 10 randomly selected plants per plot. Fresh weight per plant was recorded by individually weighing the same 10 plants used for leaf measurements, including both aboveground biomass and the hypocotyl.

For agronomic yield estimation, all hypocotyls from the central harvest area of each plot (8 × 3 m, excluding a 1 m border on each side) were manually collected. The harvested material was weighed in the field to obtain total fresh weight per plot. A representative subsample of approximately 500 g per plot was oven-dried at 65 °C to constant weight to determine moisture content. Final yield was expressed in kilograms per hectare (kg ha^{-1}) of dry matter, adjusted to a standard moisture content of 12%.

2.6.2. Nutritional quality and energy value

Harvested *L meyenii* hypocotyls were washed with distilled water to remove adhering soil particles, oven-dried with forced air circulation at 65 °C until constant weight (approximately 72 h), ground using a knife mill (0.5 mm mesh), and stored in airtight glass containers at room temperature until analysis.

Chemical–nutritional characterization was performed following standardized methods. Total ash content was determined by incineration in a muffle furnace at 550 °C for 4 h until white or grayish ash was obtained, according to AOAC method 930.05 (Latimer Jr., 2023). Crude protein was quantified using block digestion with concentrated sulfuric acid and copper sulfate as a catalyst, followed by steam distillation into boric acid, in accordance with AOAC method 2011.11 (Latimer Jr., 2023). Total nitrogen was multiplied by a conversion factor of 6.25 to express crude protein content. Ether extract (crude fat) was determined using the gravimetric AOAC method 930.09, employing petroleum ether as solvent in a Soxhlet apparatus with 6 h of continuous extraction. Crude fiber was evaluated following AOCS method Ba 6-84 using an Ankom crude fiber analyzer equipped with ceramic filters (SOCIETY, 2017).

Total carbohydrates were estimated by difference using the following formula:

$$\text{Carbohydrates}\,(\%) = 100 - (\text{ash} + \text{crude protein} + \text{crude fat} + \text{crude fiber})$$

Total energy value was calculated using the standard Atwater conversion factors (4 kcal g for protein and carbohydrates, 9 kcal g^{-1} for fat), as described by Collazos (1993). All results for ash, crude fat, crude protein, crude fiber, and carbohydrates were expressed as g 100 g^{-1} dry matter, whereas total energy value was expressed as kcal 100 g^{-1}.

2.6.3. Macro- and micronutrient composition

Previously dried and ground hypocotyl samples (Section 2.6.2) were subjected to microwave-assisted acid digestion following EPA Method 3051A (U.S. EPA, 2007). Briefly, 0.5 g of sample was weighed into Teflon vessels, and 10 mL of concentrated HNO_3 (reagent grade, 65%) was added. Samples were digested using a Milestone Ethos Up microwave system under the following program: temperature ramp to 180 °C over 15 min, holding at 180 °C for 20 min, followed by cooling to room temperature. The resulting digests were transferred to volumetric flasks and brought to a final volume of 25 mL with ultrapure water (18.2 MΩ·cm).

Elemental quantification was performed using Microwave Plasma–Atomic Emission Spectroscopy (MP-AES) with an Agilent 4200 MP-AES system, following the operating conditions recommended by the manufacturer and the guidelines described by Balaram (2020) for applications in plant samples. Concentrations of the macronutrients calcium (Ca), magnesium (Mg), and potassium (K), as well as the micronutrients iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn), were determined.

For analytical quality control, reagent blanks were included in each digestion batch ($n = 3$), and duplicate samples (10% of the total) were analyzed. Method precision and accuracy were verified through analysis of the certified reference material NIST SRM 1515 (apple leaves), with recoveries falling within the acceptable range (90–110%) for all elements. Method detection limits (MDLs) for each element were as follows: Ca 0.5, Mg 0.2, K 0.8, Fe 0.1, Zn 0.05, Cu 0.02, and Mn 0.03 mg kg^{-1}. All results were expressed as mg kg^{-1} on a dry matter basis.

2.6.4. Hypocotyl color classification

Following harvest and washing, a random subsample of 130 hypocotyls was collected from each experimental unit. Each hypocotyl was visually classified according to its predominant external pigmentation into one of three color categories: purple, black, or yellow. The number of hypocotyls belonging to each category was recorded, and the relative frequency of each color type was expressed as a percentage of the total sampled hypocotyls per plot.

The evaluated color categories correspond to commonly recognized maca phenotypes traditionally distinguished by hypocotyl pigmentation. Representative images of the evaluated color classes are provided in Supplementary Fig. S1.

2.7. Statistical analysis

Data were analyzed under a randomized complete block design (RCBD) with four blocks. For continuous variables, including morphological traits, productive parameters, nutritional composition, hypocotyl mineral content, and post-harvest soil chemical properties (soil pH, electrical conductivity, and available K), analysis of variance (ANOVA) was performed using the aov function in R (R Core Team, 2021), considering treatments as fixed effects and blocks as blocking factors. Prior to analysis, model assumptions were verified by assessing the normality of residuals using the Shapiro–Wilk test and the homogeneity of variances using Levene's test. When significant treatment effects were detected ($p < 0.05$), multiple comparisons were performed using Tukey's honestly significant difference (HSD) test implemented in the agricolae package.

Hypocotyl color proportions (categories: purple, black, and yellow) were analyzed using generalized linear models (GLMs) with a multinomial distribution, implemented through the multinom function in the nnet package. The overall significance of treatment effects on color distribution was assessed using likelihood ratio tests (function lrtest, lmtest package).

Additionally, the relationship between the proportion of black hypocotyls and dry matter yield was explored using simple linear regression.

For multivariate analysis, a Principal Component Analysis (PCA) was performed using the factoextra package to visualize the association between treatments and the macro- and micronutrient profile. Complementarily, a Redundancy Analysis (RDA) was conducted using the vegan package to assess the relationship between treatments (explanatory variables) and the set of response variables (productive, nutritional, and mineral traits). Prior to RDA, variables were standardized (mean = 0, standard deviation = 1) to ensure comparability across scales. The significance of canonical axes and of the overall model was evaluated using permutation tests (999 permutations) with the anova.cca function.

The overall nutritional profile was represented using a radial (spider) plot generated with the fmsb package, following Min–Max normalization (0–1) of the variables. All visualizations were produced using the ggplot2, ggpmisc, patchwork, and scales packages. The level of significance adopted for all analyses was $\alpha = 0.05$.

The R code used for the analyses is available from the authors upon reasonable request.

3. Results

3.1. Morphological characteristics: plant establishment and leaf growth

Significant differences were observed in the morphological traits evaluated following the application of organic and biological amendments on the development of *L. meyenii* (Fig. 2). Regarding plant establishment (Fig. 2A), the biochar treatment showed the highest mean density, with approximately 49 plants m^{-2}, exhibiting a statistically significant difference ($p < 0.05$) compared with the control, which recorded the lowest plant density (29 plants m^{-2}). The biochar + *B. subtilis* and liquid *B. subtilis* treatments showed intermediate values of 44 and 39 plants m^{-2}, respectively, and did not differ significantly from the biochar-only treatment.

Regarding leaf size (Fig. 2B), a clear and consistent response to biofertilization was observed. All evaluated treatments (biochar + *B. subtilis*, biochar and *B. subtilis*) significantly outperformed the control group ($p < 0.05$). While the control exhibited an average leaf length of 9.7 cm, the treated groups reached values exceeding 14 cm. Notably, no significant differences were detected among the three biofertilizer treatments, suggesting that both the porous support provided by biochar and the bacterial activity promote leaf expansion to a similar extent under the abiotic stress conditions of the Puna. These results position these treatments as effective promoters of early morphological development in maca cultivation.

3.2. Productive traits: biomass accumulation and dry matter yield

Yield parameters showed highly significant statistical differences among treatments, highlighting the superiority of the combined

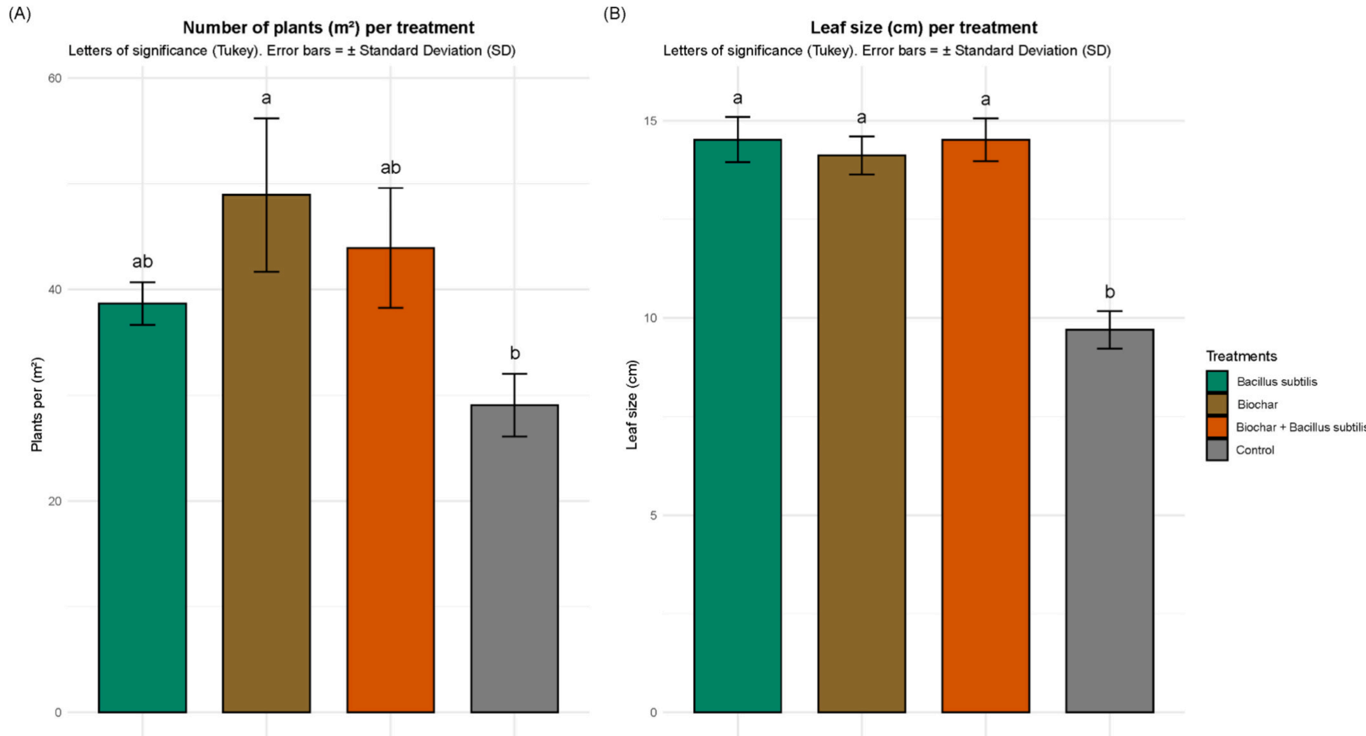

Fig. 2. Agronomic response of maca to a biofertilizer based on *B. subtilis* immobilized on guinea pig manure–derived biochar.

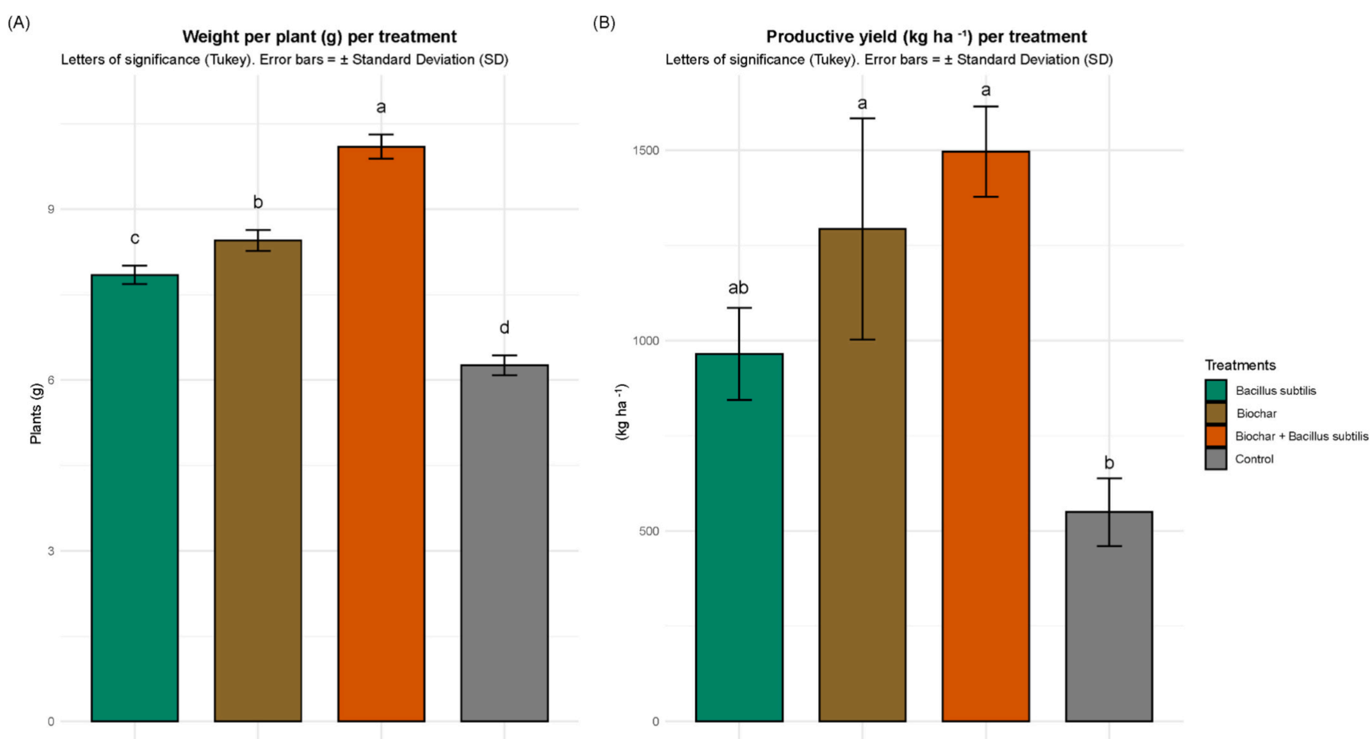

Fig. 3. Effects of microbiological and organic fertilization on morphological parameters of *L. meyenii*.

biological and organic amendment (Fig. 3). Plant weight (Fig. 3A) displayed a clear hierarchy, with the biochar + *B. subtilis* treatment reaching the highest values (10.1 g), significantly outperforming the individual biochar and *B. subtilis* treatments. The control group recorded the lowest individual plant weight (6.2 g), indicating that the integration of the microorganism–support system enhances biomass accumulation more effectively than either component applied separately.

Regarding crop yield (kg ha^{-1}), a similar trend of substantial improvement due to biofertilization was observed. The biochar + *B. subtilis* and biochar-only treatments led productivity, forming the upper performance group with yields reaching 1500 kg ha^{-1} and 1290

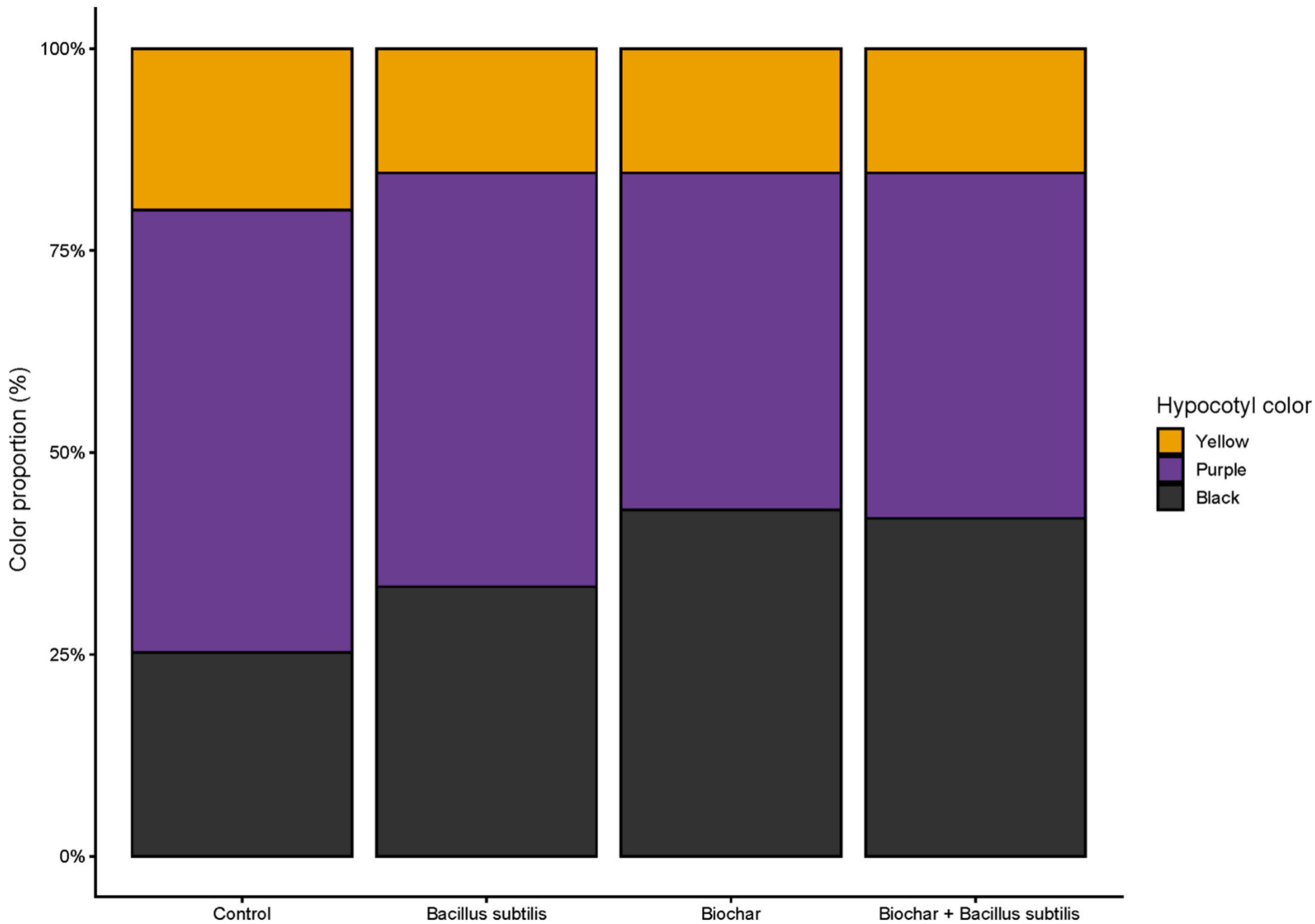

Fig. 4. Distribution of hypocotyl color (%) under different treatments. A significant treatment effect was detected (likelihood ratio test: $\chi^2 = 61.41$, p < 0.001).

kg ha^{-1} of dry matter, respectively. Both treatments more than doubled the yield of the control group, which recorded the lowest value at approximately 550 kg ha^{-1}. The liquid *B. subtilis* treatment showed an intermediate yield (960 kg ha^{-1}), suggesting that although the bacterium alone improves productivity, its effectiveness is maximized when applied together with guinea pig manure–derived biochar. These results confirm that the use of immobilized biofertilizers represents an effective strategy to increase maca productivity under conditions of high abiotic stress.

3.3. Hypocotyl color distribution

The distribution of hypocotyl color was significantly affected by the treatment (likelihood ratio test: $\chi^2 = 61.41$, $p < 0.001$) (Fig. 4). In the control treatment, purple was the predominant color (55%), followed by black (25%) and yellow (20%). Inoculation with *B. subtilis* increased the proportion of black hypocotyls (33%) and slightly reduced the yellow fraction (15%), while purple remained the dominant category (52%).

The biochar treatment showed a higher proportion of black hypocotyls (43%) compared with the control, accompanied by a relative decrease in purple (42%) and yellow (15%). Similarly, the combined biochar + *B. subtilis* treatment exhibited a high proportion of black hypocotyls (42%), with intermediate values for purple (43%) and a lower fraction of yellow (15%).

The combined application of biochar and *B. subtilis* increased the relative frequency of black hypocotyl, whereas the control treatment showed a greater proportion of purple individuals.

In addition to the treatment effect on hypocotyl color distribution, a significant positive association was observed between the proportion of black hypocotyls and dry matter yield ($R^2 = 0.20$, $p = 0.047$) (Fig. 5). Dry matter production increased by approximately 23 kg ha^{-1} for each 1% increase in the frequency of black hypocotyls. No significant relationship was detected between the proportion of purple hypocotyls and dry matter yield ($p > 0.05$).

In general, higher proportions of black hypocotyls were associated with increased yield, with values above 40% linked to yields exceeding 1200 kg ha^{-1} However, the dispersion of the data and the moderate R^2 value indicate a relationship of low to intermediate magnitude. These results therefore suggest that the proportion of black hypocotyls was positively associated with the productive performance of the crop under the evaluated conditions.

3.4. Nutritional quality of maca hypocotyls

The analysis of variance revealed that treatment exerted a significant effect on the protein and fat content of the hypocotyl (Table 1). Protein content was significantly influenced by the treatments ($F = 5.65$; $p = 0.019$), whereas the block effect was not significant ($p = 0.618$). Similarly, fat content showed significant differences among treatments ($F = 8.29$; $p = 0.006$), although a significant block effect was also detected ($F = 4.26$; $p = 0.039$).

In contrast, ash content showed a tendency to differ among treatments ($F = 3.00$; $p = 0.088$), with no significant block effect detected. No significant differences were observed for crude fiber, carbohydrates, or

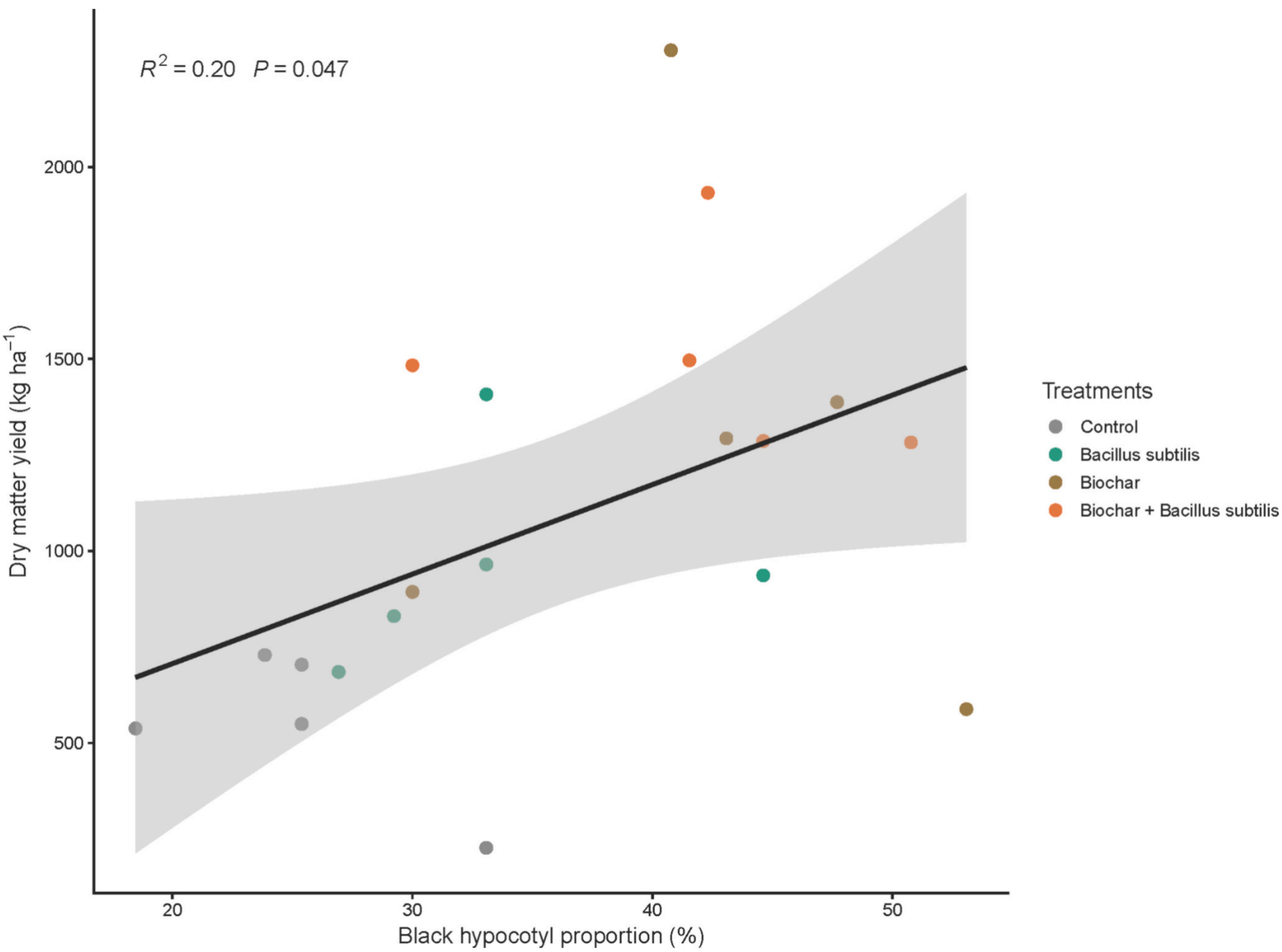

Fig. 5. Relationship between the proportion of black hypocotyls (%) and dry matter yield (kg ha^{-1}). The solid line represents the overall linear regression ($R^2 = 0.20$, $p = 0.047$).

Table 1
Analysis of variance (ANOVA) for the nutritional quality of the hypocotyl under a randomized complete block design (RCBD).

Variable	df	F treatment	p treatment	F block	p block
Protein (g 100 g^{-1})	3	5.65	0.019*	0.62	0.618
Ash (g 100 g^{-1})	3	3.00	0.088	1.22	0.359
Fat (g 100 g^{-1})	3	8.29	0.006**	4.26	0.039*
Crude fiber (g 100 g^{-1})	3	0.30	0.827	0.65	0.603
Carbohydrates (g 100 g^{-1})	3	0.56	0.657	1.69	0.238
Total energy (kcal 100 g^{-1})	3	0.90	0.477	1.97	0.189

*Values correspond to F statistics obtained from ANOVA. Significance levels: *$p < 0.05$; **$p < 0.01$; **$p < 0.001$.

total energy ($p > 0.05$), and these variables were likewise unaffected by the block factor. Overall, the results indicate that the evaluated treatments primarily influenced the protein and lipid components of the hypocotyl, whereas the remaining nutritional parameters remained relatively stable under the experimental conditions.

Based on these results, a Tukey multiple comparison test was performed to identify specific differences among treatments for protein and fat contents (Table 2). For protein content, the *B. subtilis* treatment showed the highest mean value, differing significantly from the biochar + *B. subtilis* treatment ($p < 0.05$), whereas the control and biochar treatments exhibited intermediate values without clear statistical differences.

To determine whether the observed differences in protein concentration translated into changes in absolute protein production, protein yield per hectare was calculated as the product of dry matter yield and protein concentration. Protein yield ranged from 20.18 kg ha^{-1} in the control treatment to 49.78 kg ha^{-1} in the combined biochar + *Bacillus subtilis* treatment, with intermediate values recorded for biochar (44.24 kg ha^{-1}) and *B. subtilis* (37.43 kg ha^{-1}). However, treatment effects were not statistically significant ($p = 0.104$; Tables S11 and S12), indicating that differences in protein concentration among treatments did not result in significant differences in absolute protein production per unit area.

Regarding fat content, the biochar treatment recorded significantly lower values compared with *B. subtilis* and the control ($p < 0.05$), whereas the combined treatment exhibited an intermediate response.

Table 2
Effect of treatments on hypocotyl protein and fat content.

Treatment	Protein (g 100 g^{-1})	Fat (g 100 g^{-1})
Control	3.61 ± 0.29^{ab}	0.22 ± 0.05^{a}
Biochar	3.44 ± 0.05^{ab}	0.18 ± 0.04^{b}
Bacillus subtilis	3.88 ± 0. 17^{a}	0.24 ± 0.04^{a}
Biochar + *Bacillus subtilis*	3.34 ± 0.16^{b}	0.22 ± 0.02^{ab}

Values are expressed as mean ± standard deviation. Different letters within each column indicate significant differences according to Tukey's test ($p < 0.05$).

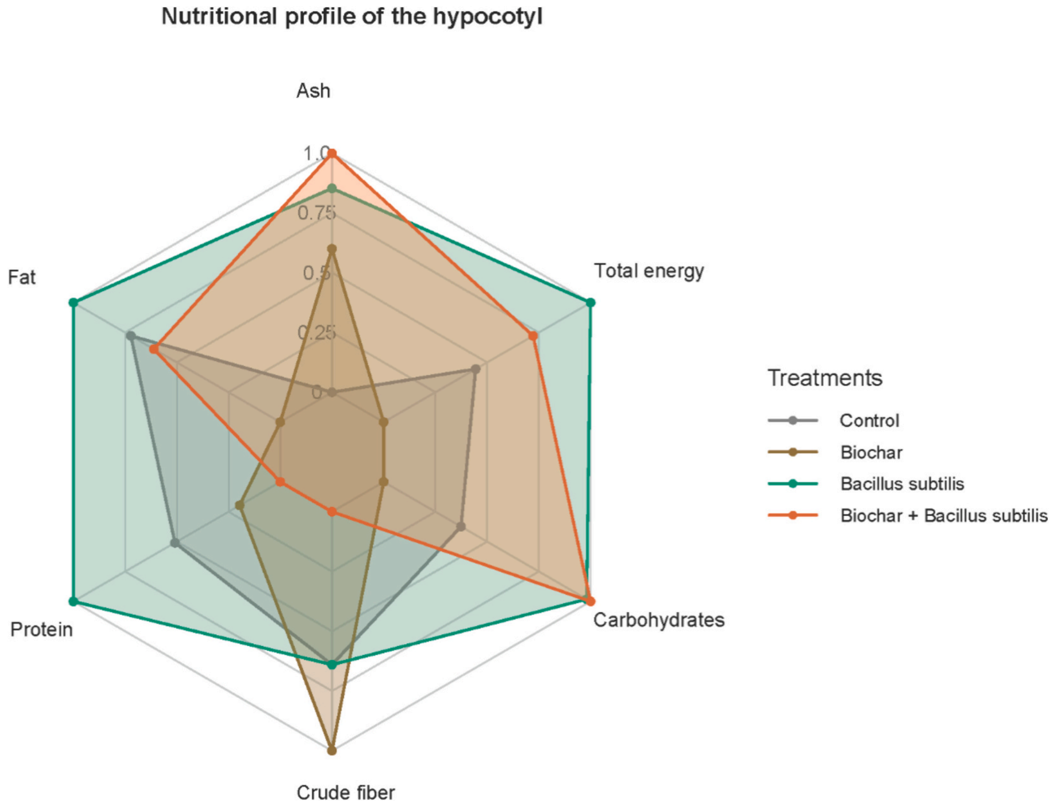

Fig. 6. Nutritional profile of hypocotyls as affected by treatment.

To comprehensively visualize the variations observed in the proximate composition, a comparative analysis of the nutritional profile of the hypocotyl was performed (Fig. 6), considering the normalization of the variables ash, fat, protein, crude fiber, carbohydrates, and total energy. This approach allows the identification of relative trends and response patterns among treatments. In general, the treatment with *B. subtilis* exhibited the most balanced and overall higher nutritional profile across most of the evaluated components, particularly in protein, total energy, carbohydrates, and fat (Fig. 6). This pattern suggests a positive effect of the microbial inoculant on nutrient assimilation and accumulation, possibly associated with improved nutrient availability and enhanced metabolic efficiency in the plant.

In contrast, the biochar treatment exhibited a more restricted nutritional profile, with relatively lower values for protein, carbohydrates, and total energy, although showing a tendency toward higher crude fiber levels compared with the other treatments. This pattern may indicate that biochar preferentially promotes structural aspects of plant tissues rather than the accumulation of energy-rich or nitrogen-containing compounds.

In the combined treatment (biochar + *Bacillus subtilis*), the nutritional profile showed noticeable increases in ash and carbohydrates, while maintaining intermediate fat values, consistent with those reported in Table 2, and moderate levels of protein and crude fiber. Although the combined treatment showed improvements compared with biochar alone, it did not consistently outperform the treatment with *B. subtilis* applied individually.

The multivariate analysis suggests that inoculation with *B. subtilis* exerted the most favorable effect on the nutritional quality of the hypocotyl, whereas biochar showed more specific and less extensive effects. The interaction between both factors did not demonstrate a generalized synergistic effect across all evaluated components, indicating that the nutritional response depends on the type of amendment applied and its influence on soil–plant dynamics.

Table 3
Analysis of variance (ANOVA) for macro- and micronutrient content of the hypocotyl under a randomized complete block design (RCBD).

Variable (mg kg^{-1})	df	F treatment	p treatment	F block	p block
Macronutrients					
Ca	3	1.42	0.301	0.46	0.718
Mg	3	3.86	0.050	0.91	0.475
K	3	24.68	0.0001***	0.29	0.835
Micronutrients					
Fe	3	2.93	0.092	1.89	0.202
Zn	3	1.37	0.313	0.15	0.926
Cu	3	2.75	0.104	1.07	0.409
Mn	3	0.17	0.917	0.65	0.603

Values correspond to F statistics obtained from ANOVA. Significance levels: *p < 0.05; **p < 0.01; **p < 0.001.

3.5. Macro- and micronutrient content (Ca, Mg, K, Fe, Zn, Cu and Mn)

The analysis of variance revealed that the soil treatment significantly influenced the K content in the hypocotyl ($p < 0.001$), whereas a marginal trend was observed for Mg ($p = 0.050$). In the case of Fe, a statistical tendency was detected ($p < 0.10$). No significant treatment

Table 4
Mean comparison (Tukey, $p < 0.05$) for Mg and K in the hypocotyl.

Treatment	Mg (mg kg^{-1})	K (mg kg^{-1})
Control	1501.33^{b}	13,601.67^{c}
Biochar	1983.67^{ab}	22,252.00^{a}
Bacillus subtilis	1977.67^{ab}	17,211.33^{b}
Biochar + *B. subtilis*	2250.67^{a}	20,862.33^{a}

Values are expressed as mean ± standard deviation. Different letters within each column indicate significant differences according to Tukey's test (p < 0.05).

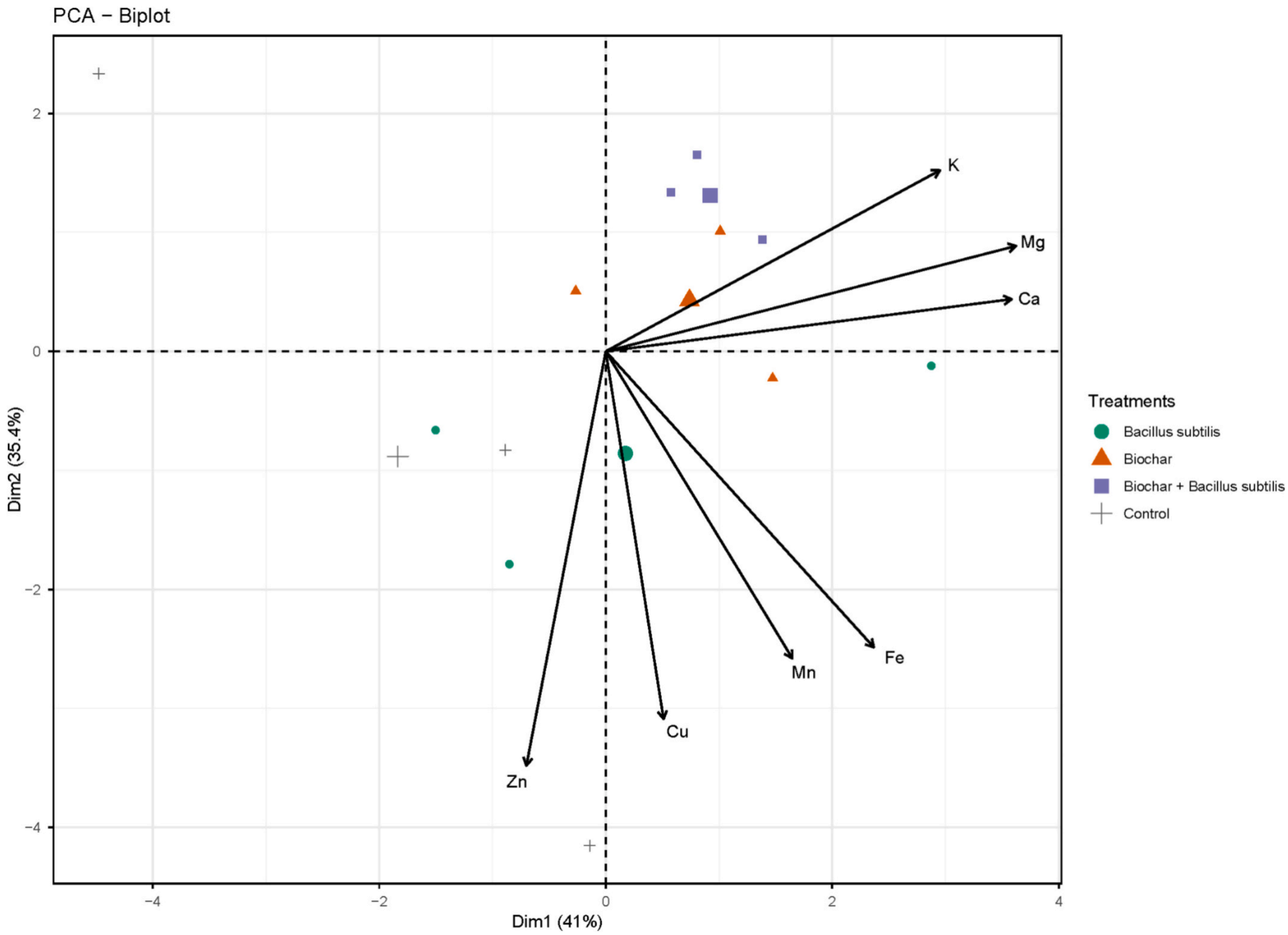

Fig. 7. Principal component analysis (PCA) of hypocotyl macro- and micronutrient composition (Ca, Mg, K, Fe, Zn, Cu, and Mn) as affected by edaphic treatments.

effects were found for Ca, Zn, Cu, or Mn ($p > 0.05$). The block factor did not show significant effects for any of the evaluated nutrients, indicating adequate experimental homogeneity (Table 3).

The multiple comparison analysis (Tukey test) revealed significant differences in K content among treatments (Table 4). The biochar treatments, both applied individually and in combination with *B. subtilis*, exhibited the highest K values, significantly exceeding those observed in the individual inoculation treatment and the control. The control treatment recorded the lowest content of this macronutrient.

In the case of Mg, the combined treatment showed the highest values and differed significantly from the control, whereas the biochar and *Bacillus subtilis* treatments applied individually exhibited intermediate values. These results suggest that biochar plays a key role in the availability and accumulation of potassium, while its combination with the inoculant may enhance magnesium uptake.

To evaluate the overall behavior of the mineral profile of the hypocotyl, a Principal Component Analysis (PCA) was performed. The first two dimensions (Dim1 and Dim2) explained 76.4% of the total variance (41% and 35.4%, respectively), indicating a high level of data representativeness (Fig. 7).

The biplot reveals a clear separation among the evaluated groups. Treatments including biochar (applied alone or in combination with *B. subtilis*) were predominantly positioned in the upper-right quadrant, showing a strong positive correlation with the macronutrients K, Mg, and Ca. This association suggests that the application of guinea pig manure–derived biochar significantly enhances the translocation of essential cations to the maca hypocotyl.

In contrast, micronutrients such as Fe, Mn, and Cu were projected toward the lower-right quadrant, showing a closer association with some replicates of the *B. subtilis* treatment. Notably, the control group was positioned opposite to the vectors of most nutrients (left quadrants), confirming a poorer mineral profile compared with biofertilized plants. Zinc (Zn) exhibited a distinct pattern, orienting toward the lower-left quadrant, which may indicate a different uptake dynamic for this microelement under the conditions of the present study.

3.6. Post-harvest soil chemical properties

Post-harvest soil analyses revealed significant treatment effects on soil pH and available K, whereas EC was not significantly affected (Table S1). Biochar-containing treatments exhibited higher soil pH values (5.23–5.60) than the control and *B. subtilis* treatments (4.50–4.60). Similarly, available K was markedly greater in soils amended with biochar, particularly in the combined biochar + *B. subtilis* treatment (541.72 mg kg^{-1}), compared with the initial soil concentration (111.33 mg kg^{-1}) and the non-biochar treatments. These results indicate that biochar-based amendments increased K availability and partially mitigated soil acidity without causing a significant increase in soil salinity.

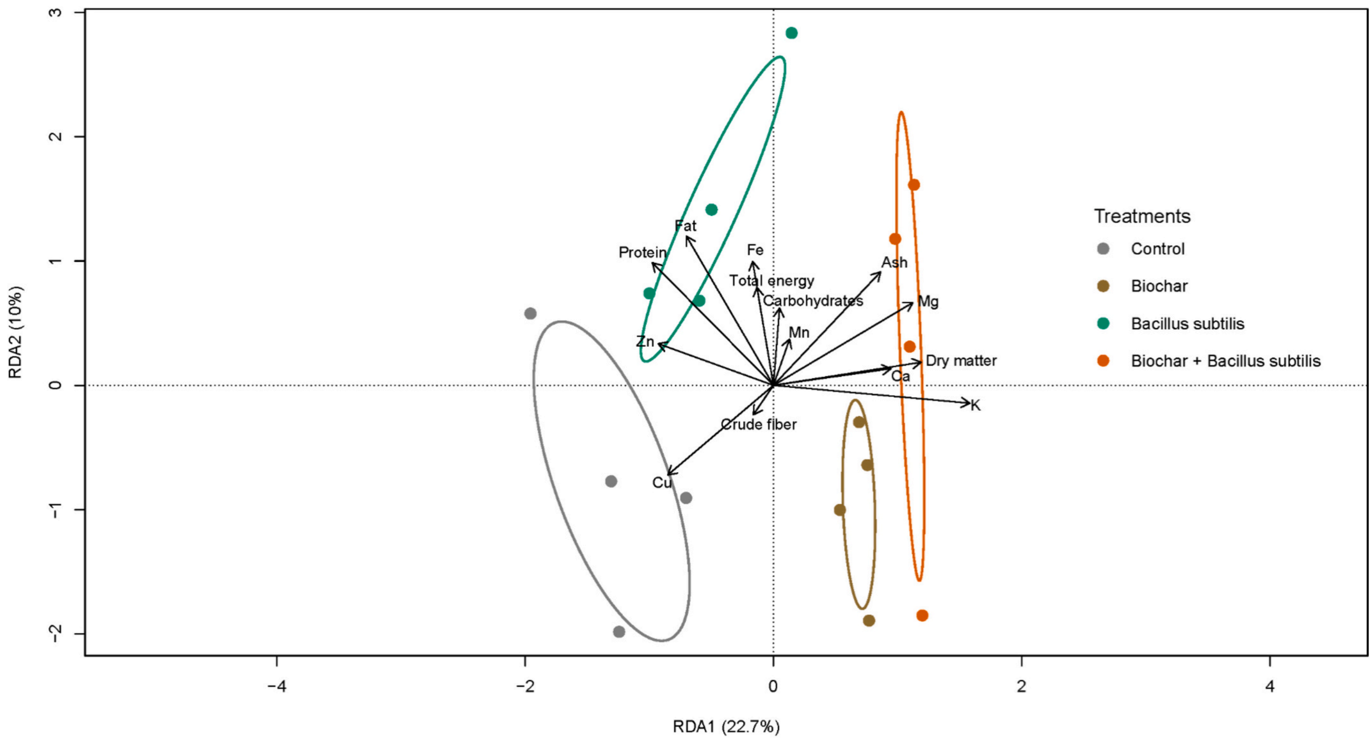

Fig. 8. Redundancy analysis (RDA) of nutritional, mineral, and productive profiles in response to edaphic treatments.

3.7. Explanatory multivariate analysis of response variables under edaphic treatments

The redundancy analysis (RDA) explained 32.7% of the total joint variation in the mineral, nutritional, and productive profile under the evaluated soil treatments, with RDA1 accounting for 22.7% and RDA2 for 10.0% (Fig. 8). This level of explained variance is moderate and expected under field conditions, where a substantial proportion of variation is typically driven by factors not included in the model. Nevertheless, the ordination revealed a consistent structuring of the treatments, with a clear separation between the control and the amended treatments, indicating that the treatments captured a relevant component of the variability in the response variables.

The lower dispersion of replicates observed in the biochar treatments and in the combined treatment suggests a more consistent response among replicates compared with the control, which exhibited greater dispersion.

In functional terms, RDA1 defined a mineral–productive gradient, with positive loadings dominated by K, Ca, Mg, ash, and dry matter, which displayed the longest vectors and therefore the greatest contribution to discrimination along this axis. Treatments with biochar (alone and in combination with *Bacillus subtilis*) were positioned predominantly toward the positive quadrant of RDA1, suggesting a close association between the organic amendment and higher cation density and productive performance. In contrast, RDA2 structured a more nutritional gradient, where protein, fat, Fe, Zn, and total energy projected toward the upper left quadrant and were spatially associated with the treatment inoculated with *B. subtilis*, evidencing a distinct proximate composition profile compared with treatments dominated by biochar. Variables such as carbohydrates showed an intermediate association, whereas Mn presented a short vector located near the origin, indicating low discriminatory power in this experiment. Overall, the RDA supports an interpretation of functional complementarity between amendments: biochar is primarily associated with the mineral–productive component, whereas *B. subtilis* is linked to the proximate–micronutritional component.

4. Discussion

4.1. Morphological characteristics: plant establishment and leaf growth

The higher plant density of maca observed in the biochar treatment compared with the control suggests that the carbonized amendment primarily acted on the edaphic filter that regulates plant establishment in high-Andean systems. This effect likely translated into improved water retention, increased nutrient availability, and partial buffering of edaphic acidity, factors that collectively promote seedling emergence and early survival (Jatuwong et al., 2025). In addition, guinea pig manure-derived biochar typically exhibits an alkaline pH and a mineral-rich composition, properties that can help neutralize acidic soils and create a more stable edaphic environment for early plant development (Bolan et al., 2023; Huang et al., 2023; Solórzano, 2026).

The consistently greater leaf length observed in the three biofertilizer treatments (>14 cm) compared with the control suggests an improved nutritional and physiological status of maca during the early vegetative stage. Bacterial inoculants, including *Bacillus*, can promote leaf expansion by enhancing nutrient acquisition, modulating phytohormonal signaling, and improving tolerance to abiotic stress. For instance, *Bradyrhizobium japonicum* and *B. subtilis* have been reported to stimulate root growth and alter root architecture, thereby increasing the capacity for nutrient uptake in legumes (Fernando et al., 2021). Similarly, Jang et al. (2018) showed that *B. subtilis* increased photosynthetic rate and foliar nitrogen content, resulting in improved nutrient use efficiency in *Populus euramericana* and *Populus deltoides* × *P. nigra*. In parallel, biochar may further enhance these responses by modifying the microbial habitat and nutrient dynamics in the rhizosphere (Huang et al., 2023; Siddika et al., 2024).

The absence of significant differences among the, biochar + *B. subtilis and* liquid *B. subtilis* treatments for leaf length suggests that, once the primary edaphic constraints (e.g., water and nutrient availability) are alleviated, leaf expansion may become regulated by other factors such as genotype, microclimatic conditions, or crop phenology, leading treatments to converge toward a similar performance. Moreover, the persistence and establishment of microbial inoculants under field conditions can be variable. For this reason, immobilization strategies using biochar have been proposed to enhance microbial survival, protection, and gradual release in the rhizosphere, although their differential benefits may become more evident in later variables such as biomass accumulation, mineral uptake, and yield (Vlajkov et al., 2023; Yu et al., 2025). These morphological responses provide a preliminary framework for understanding the functional divergence that becomes more clearly expressed in the productive and nutritional variables discussed in the following sections.

4.2. Productive traits: biomass accumulation, fresh yield, and dry matter yield

Evidence suggests that biochar and *B. subtilis* can interact synergistically to improve plant performance. For example, Liang et al. (2024) reported greater individual plant weight when both inputs were applied together under abiotic stress conditions. However, the present study was conducted under natural rainfed field conditions without experimentally imposed stress. Therefore, direct comparisons should be interpreted with caution, as the mechanisms underlying plant responses may differ between stressed and non-stressed environments. This effect is generally attributed to the combined action of biochar-mediated improvements in soil properties and the plant growth-promoting (PGP) functions of *Bacillus*, which together enhance resource acquisition and biomass accumulation. The pattern observed here, where the combined treatment outperformed the bacterial inoculant applied alone, is consistent with findings by Sifton et al. (2023), who demonstrated that the co-application of biochar and biofertilizers improves nutrient uptake and nutrient use efficiency. Immobilization of microbial inoculants in porous carbonaceous matrices is particularly advantageous because biochar provides microhabitats and adsorption surfaces that protect cells or endospores and facilitate a more sustained release of the inoculant, thereby increasing the likelihood of effective root colonization (Vlajkov et al., 2023; Nakahara et al., 2025). Tripti et al. (2016) reported that biochar prolonged the survival of plant growth-promoting rhizobacteria (*Burkholderia* sp. strain L2 and *Bacillus* sp. strain A30), leading to increased biomass and yield in tomato The use of biochar as a microbial carrier enhances the survival and colonization of inoculants in the soil and plant roots, playing a crucial role in soil biochemical processes and nutrient and carbon cycling (Bolan et al., 2023). Besides providing physical protection and adsorption sites for microbial cells, the carrier formulation itself may also influence microbial activity in the soil. In the present study, molasses was incorporated during the immobilization process to facilitate the attachment of *Bacillus subtilis* to the biochar matrix; however, it represented only a small fraction of the final formulation (approximately 0.21% v/v). Although readily available carbon sources such as molasses may transiently stimulate microbial metabolism following application, the superior performance of the combined treatment is more likely associated with the interaction between the biochar carrier and the inoculated bacterium. Nevertheless, a minor contribution from formulation-associated effects cannot be completely excluded, as interactions among carrier materials, carbon sources, and microbial inoculants are recognized components of biofertilizer systems under field conditions.

Regarding dry matter yield, the fact that biochar alone achieved a high productivity indicates that the carbon-rich organic amendment is already acting as a primary driver of the edaphic environment The microbial inoculant appears to enhance this effect when its activity is sustained throughout the crop cycle. In agricultural trials, the integration of biochar with organic amendments has been shown to increase crop productivity and improve indicators of soil fertility and nutrient availability, demonstrating that the carbonaceous and mineral components of biochar can exert direct productive effects beyond those of the inoculant (Liang et al., 2024). The advantage observed in the biochar + *B. subtilis* treatment is consistent with a scenario in which biochar also functions as a microbial carrier that maintains functional microbial activity during the crop cycle, thereby amplifying the conversion of nutrients into plant biomass (Saldarriaga and López, 2025; Sharma et al., 2025). This integrated approach is particularly relevant in monocropping systems such as maca, where soil degradation and the need to optimize management practices are recognized constraints to sustaining productivity Notably, these yield responses—driven mainly by the biochar-based treatments—contrast with the patterns observed for nutritional quality, which are discussed in the following sections and highlight the functional divergence that constitutes the central finding of this study.

4.3. Hypocotyl morphological characteristics

The distribution of hypocotyl color showed a higher proportion of black hypocotyls in the biochar treatments, which may be attributed to the capacity of biochar to create a more favorable edaphic microenvironment. This pattern also coincided with the higher plant density observed in these treatments. Although the black hypocotyl phenotype has been reported to exhibit more restrictive germination under certain conditions (Janampa-Santome et al., 2021), our results suggest that biochar may mitigate these limitations by improving soil conditions.

Evidence indicates that hypocotyl color in maca is associated with its biological activity and represents a phenotypic trait linked to genetic variability among recognized maca ecotypes (Gonzales et al., 2006; Gonzales and Gonzales-Castaneda, 2009; Yabar et al., 2011; Gonzales et al., 2014). Previous studies have reported differences among black, purple, and yellow maca phenotypes in phytochemical composition and functional properties, including variations in glucosinolate content, phenolic compounds, antioxidant activity, and other bioactive metabolites. Consequently, shifts in hypocotyl color distribution may reflect differential physiological responses to environmental conditions and soil management practices.

Pigmentation is also linked to physical and chemical seed traits that modulate water imbibition, oxygen diffusion, and dormancy processes. These characteristics are frequently associated with the presence of phenolic compounds and proanthocyanidins, which increase seed coat hardness and impermeability, potentially delaying the emergence of darker hypocotyls (Debeaujon et al., 2000; Atis et al., 2011; Ertekin and Kirdar, 2010). In maca, the presence of glucosinolates has been documented in seeds and other tissues, and their hydrolysis products (isothiocyanates) may exert inhibitory effects on germination. Consequently, greater pigmentation could coexist with higher concentrations of restrictive secondary metabolites, particularly under stress conditions (Li et al., 2001; Brown and Morra, 1996; Haramoto and Gallandt, 2004).

In this context, biochar may favor the occurrence of black hypocotyls by improving soil water retention, buffering soil pH, and adsorbing or attenuating inhibitory compounds through sorption processes at the seed–soil interface, thereby reducing physiological constraints during imbibition and radicle protrusion (Razzaghi et al., 2020; Chen et al., 2022; Bolan et al., 2023). In this study, the higher proportion of black hypocotyls was positively associated with dry matter yield ($R^2 = 0.20$, $p = 0.047$; Fig. 5), suggesting that the soil conditions generated by biochar application may favor the occurrence of this phenotype under field conditions. Although the underlying mechanisms remain unclear, the observed association indicates that changes in hypocotyl color distribution may constitute an additional response of maca populations to soil amendments. Because hypocotyl color was not experimentally manipulated, this relationship should be interpreted as an association rather than evidence of a direct causal effect.

4.4. Nutritional quality of maca hypocotyls

The higher protein content observed in the *B. subtilis* treatment alone (3.88 g 100 g^{-1}; Table 2) is consistent with the role of *Bacillus* as PGPR capable of enhancing nitrogen acquisition and metabolic efficiency, thereby stimulating the synthesis of nitrogen-containing compounds (Fonseca et al., 2022; Patel et al., 2023). In this context, bacterial inoculants have been reported to improve nutrient availability and uptake while reshaping rhizosphere processes, with effects that can be reflected in the nutritional quality of harvested tissues, particularly when the production system is constrained by soil fertility or environmental stress (Solórzano-Acosta and Quispe, 2024; Fortu et al., 2025; Díaz-Rodríguez et al., 2025). The absence of a clear synergistic effect of the combined treatment on nutritional quality—where the lowest protein content was recorded (3.34 g 100 g^{-1}), —suggests that the synergy between biochar and *Bacillus* may be expressed primarily in yield performance and mineral accumulation rather than in all proximal components. This interpretation is reinforced by the protein yield analysis, which showed that the combined treatment produced the highest numerical protein yield per hectare (49.78 kg ha^{-1}), despite exhibiting the lowest protein concentration. Therefore, the reduction in protein concentration was compensated by greater dry matter production, resulting in comparable or higher absolute protein output per unit area. Such patterns are common when the limiting factor shifts among treatments (Bolan et al., 2023; Jiali et al., 2025).

The lower lipid content observed in the biochar-only treatment (0.18 g 100 g^{-1}; Table 2) together with the intermediate response of the combined treatment, may reflect a reallocation of resources toward biomass production at the expense of lipid accumulation. Alternatively, it could result from shifts in relative nutrient availability that prioritize vegetative growth over the synthesis of lipid compounds (García Gómez et al., 2024; Adekiya et al., 2025). Studies where biochar and microbial inoculants are applied together have shown that improvements in soil conditions can increase crop productivity while also modifying tissue composition, including protein content; however, the direction and magnitude of these changes depend on the type of biochar, the crop species, and the level of environmental stress (Ma et al., 2022; Song et al., 2025). These results reinforce the proposed functional divergence: biochar alone primarily enhances productivity —often accompanied by reductions in components such as lipids and protein—, whereas *B. subtilis* alone tends to optimize nutritional quality. Their combined application does not fully integrate both benefits, revealing a trade-off between yield and quality that should be considered when designing fertilization strategies.

4.5. Macro- and micronutrient content (Ca, Mg, K, Fe, Zn, Cu and Mn)

The higher K concentrations observed in the biochar treatments (up to 22,252 mg kg^{-1}; Table 4) are consistent with the intrinsic properties of manure-derived biochars, which typically contain elevated levels of this element and enhance soil cation retention and exchange capacity (Jatuwong et al., 2025).

This interpretation is supported by the post-harvest soil analyses, which showed significantly higher soil pH and available K concentrations in the biochar-containing treatments compared with the control and *B. subtilis* treatments (Table S1). The increase in soil K availability is consistent with the high K content typically found in manure-derived biochars and may have contributed to the enhanced K accumulation observed in maca hypocotyls. In addition, the higher soil pH values suggest a partial amelioration of soil acidity, which may have favored nutrient availability and plant growth under the acidic conditions of the experimental site.

In maca production systems under continuous cultivation, where progressive soil fertility declines and nutrient limitations have been reported (Solórzano-Acosta et al., 2025), an intervention that increases available K may simultaneously explain the mineral shift observed in the RDA and part of the yield improvement. The trend observed for Mg, which was highest in the combined treatment (Table 4), is consistent with a scenario in which the organic carrier promotes nutrient availability and retention, while the microbial inoculant enhances nutrient uptake efficiency, generating a compounded effect that becomes more evident for certain cations (Wu et al., 2025).

The PCA structure (Fig. 7) provides an integrative interpretation of the nutrient patterns observed. Biochar treatments aligned primarily with K, Mg, and Ca, which represent key components of soil cation fertility, whereas *B. subtilis* was positioned closer to Fe, Mn, and Cu (micronutrients). This decoupling is consistent with a dual mechanism in which biochar modifies the soil nutrient pool and enhances the exchange and retention of base cations, while plant growth-promoting rhizobacteria (PGPR) can influence micronutrient availability through mechanisms such as solubilization, chelation, and rhizosphere-mediated transformations (Ding et al., 2016; Olanrewaju et al., 2017). The opposite positioning of the control treatment in the biplot further indicates that the amendments shift the system from a mineral-limited state toward a more favorable nutritional environment for plant performance. The clear separation between macronutrients (associated with biochar) and micronutrients (associated with *B. subtilis*) provides additional support for the proposed functional divergence: each input operates on distinct nutritional compartments, and their combined application does not necessarily integrate both benefits, but rather reflects the predominance of the biochar effect on cation accumulation.

4.6. Explanatory multivariate analysis of response variables under edaphic treatments

The redundancy analysis (RDA) explained 32.7% of the total variation in the combined mineral, nutritional, and productive profile under the evaluated edaphic treatments, with RDA1 accounting for 22.7% and RDA2 for 10.0% (Fig. 8). This proportion of explained variance can be considered moderate and is expected under field conditions, where a substantial fraction of variability is often driven by environmental factors not included in the model. Despite this, the ordination revealed a clear and consistent structure among treatments, showing a marked separation between the control and the amended treatments, indicating that the treatments captured a meaningful component of the variability in the response variables. Replicates associated with the biochar treatments (alone and combined with *B. subtilis*) showed lower dispersion, suggesting a more consistent response compared with the control, which exhibited greater variability among replicates.

Functionally, RDA1 represented a mineral–productivity gradient, with positive loadings primarily driven by K, Ca, Mg, ash, and dry matter. These variables displayed the longest vectors, indicating their strong contribution to the discrimination along this axis. Treatments including biochar (both alone and in combination with *B. subtilis*) clustered in the positive quadrant of RDA1, confirming their association with enhanced cation availability and improved crop productivity (Khan et al., 2024; Xiu et al., 2025). In contrast, RDA2 defined a nutritional–proximal gradient, where protein, fat, Fe, Zn, and total energy were oriented toward the upper-left quadrant and spatially associated with the *B. subtilis* treatment. This pattern indicates a differentiated nutritional profile compared with the treatments dominated by biochar (Hussain et al., 2025). Carbohydrates showed an intermediate position, whereas Mn displayed a short vector close to the origin, suggesting a limited discriminatory contribution within the present dataset (Anbuganesan et al., 2024; Anbuganesan et al., 2024b; Araujo et al., 2025).

The RDA provides both visual and statistical support for the proposed functional divergence between treatments: biochar was primarily associated with the mineral–productivity dimension, whereas *B. subtilis* was linked to the proximal–micronutritional dimension. This clear separation in the multivariate space indicates that both inputs operate on distinct functional pathways within the soil–plant system, resulting in a trade-off between crop productivity and nutritional quality. These findings highlight the need to consider this balance when designing fertilization strategies for maca cultivation under high-Andean conditions.

4.7. Study limitations and future research perspectives

Although the present study provides evidence that guinea pig manure–derived biochar and *Bacillus subtilis* influence maca productivity, nutritional quality, and hypocotyl color distribution, several limitations should be acknowledged. First, the survival of *B. subtilis* after field application and its colonization of the rhizosphere were not quantified. Therefore, the mechanisms underlying the differences observed between liquid and biochar-immobilized inoculation strategies cannot be directly confirmed. Future studies should assess inoculant persistence, rhizosphere colonization, and microbial population dynamics under field conditions.

Second, although soil pH, electrical conductivity, and available K were evaluated both before sowing and after harvest, intermediate measurements were not performed during the crop cycle. Consequently, the temporal dynamics of soil chemical changes and nutrient availability could not be assessed. Future studies incorporating repeated soil sampling throughout the growing season would provide a more detailed understanding of the mechanisms by which biochar and microbial inoculants influence soil processes and crop performance.

Third, although treatments containing biochar increased the proportion of black hypocotyls and this trait was positively associated with dry matter yield, hypocotyl color was not experimentally manipulated. Consequently, the observed relationship should be interpreted as an association rather than evidence of a direct causal effect. Future research should investigate the physiological and biochemical mechanisms linking maca ecotypes, hypocotyl pigmentation, and productive performance.

Finally, the experimental design did not include independent controls for molasses or carrier effects. Although molasses represented only a small proportion of the immobilized formulation (approximately 0.21% v/v) and was incorporated primarily to facilitate bacterial attachment to the biochar matrix, a minor contribution of this readily available carbon source to the observed responses cannot be completely excluded. Therefore, the individual effects of biochar, molasses, and *Bacillus subtilis* could not be fully disentangled under the present experimental design. Additional studies incorporating specific carrier controls would help clarify the relative contribution of each component and their interactions in biochar-based biofertilizer systems.

5. Conclusions

The present study demonstrates that guinea pig manure–derived biochar and *B. subtilis* inoculation exert functionally divergent effects in monoculture maca systems under high-Andean conditions, generating a trade-off between productivity and nutritional quality. Biochar application, either alone or in combination, significantly improved crop establishment and doubled dry matter yield. This effect was associated with increased accumulation of cationic macronutrients, particularly potassium, suggesting that biochar primarily functions as a soil amendment by improving edaphic properties, while also serving as a direct nutrient source, thereby promoting crop productivity. In contrast, inoculation with *B. subtilis* alone primarily promoted hypocotyl nutritional quality, resulting in the highest protein and lipid contents. Spatial associations with micronutrients such as iron and zinc further support the role of PGPB in improving the proximal composition of crops under abiotic stress conditions. The combined treatment exhibited an intermediate response: it achieved the highest individual plant weight and yields comparable to biochar alone, yet failed to capture the nutritional quality benefits observed under sole *B. subtilis* inoculation, even presenting the lowest protein content. This finding indicates that both inputs operate through largely independent primary pathways and that simple co-application does not guarantee integration of their respective benefits. Additionally, a positive relationship was identified between the proportion of black hypocotyls and yield, modulated by biochar treatments, raising new questions regarding the association among pigmentation, secondary metabolism, and responsiveness to organic soil amendments in maca. These findings provide a foundation for designing differentiated organic fertilization strategies: targeting maximum productivity through the sole application of guinea pig manure–derived biochar, or premium nutritional quality through liquid *B. subtilis* inoculation. Future research should optimize application rates and co-application strategies to harmonize both objectives, while also elucidating the molecular mechanisms underlying the color–quality–yield relationship and the interaction between biochar and native soil microbiota in long-term monoculture systems. In summary, guinea pig manure–derived biochar emerges as an effective soil amendment for restoring soils degraded by continuous maca monoculture, whereas *B. subtilis* represents a promising bioinput for the production of hypocotyls with enhanced nutritional value. Together, both contribute to the sustainability of high-Andean agriculture through complementary approaches.

CRediT authorship contribution statement

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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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2026.107259.

Data availability

Data will be made available on request.

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