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Inoculation of *Bacillus subtilis* in acidic soil amended with biochar and liming materials in maize cultivation

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The use of amendments in combination with *Bacillus subtilis* has been understudied as a strategy for rehabilitating acid soils and improving cropping systems. This study aimed to evaluate the effects of amendments and *B. subtilis* on the development, yield, and nutritional quality of the hard yellow maize Marginal 28 T variety. A randomized complete block design with a factorial arrangement was employed, considering five amendments, including biochar, alongside the application of *B. subtilis*. The combination of biochar and *B. subtilis* significantly increased plant and ear height ($p < 0.01$), achieved a grain yield of 4.11 t ha^{-1}, and reduced flowering time by seven days. Strong correlations were observed between male and female flowering ($r = 0.99$) and between stem diameter and leaf area ($r = 0.95$), indicating improved vegetative development. Soil pH and nutrient availability, such as phosphorus, were also enhanced. The combined use of amendments and *B. subtilis* optimizes yield and improves soil chemical properties. Thus, applying biochar and *B. subtilis* improves growth, yield, and soil quality, consolidating a promising strategy for sustainable agriculture in acid soils.

In Peru, approximately 50% of agricultural land is affected by high soil acidity levels, which particularly impacts production areas in the province of Alto Amazonas. In these regions, smallholder farmers often lack sustainable soil management plans, resulting in lower crop yields and reduced economic incomes[1]. Soil acidification in these areas is primarily caused by base cation leaching and, to a significant extent, by the excessive application of nitrogen fertilizers in the form of ammonium (NH_{4+}), especially in maize cultivation[2].

Hard yellow maize (*Zea mays* L.) is a strategic crop for Peru's food and economic security, supporting over 200,000 farming families across approximately 400,000 hectares and producing more than 1.2 million tons annually[3,4]. However, under humid tropical conditions with acidic soils, maize cultivation faces significant agronomic limitations due to the low availability of essential nutrients such as phosphorus, magnesium, and calcium, crucial elements for plant growth, resulting in yields of less than 2 t·ha^{-1} [5,6].

Liming has traditionally been used to counteract soil acidity; however, it presents significant limitations due to its short-term effectiveness and low mobility within the soil profile. Moreover, it does not directly enhance soil structure or contribute to other important functions such as carbon sequestration. In light of these limitations, biochar, a carbon-rich material produced through the pyrolysis of plant residues, such as rice husks, has emerged as a sustainable alternative. It has a total porosity of 76.25%, air porosity of 13.01%, moisture retention porosity of 63.23%, and a bulk density of 0.20 g·cm^{-3} [7]. Biochar improves water and nutrient retention, increases cation exchange capacity (CEC), promotes beneficial microbial activity, and contributes to climate change mitigation through long-term carbon sequestration[8,9].

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In addition, plant growth-promoting microorganisms (PGPMs), such as *Bacillus subtilis*, have demonstrated effectiveness as bioinoculants by enhancing the availability of nitrogen and phosphorus, inducing tolerance to abiotic stress, and stimulating root development through the production of phytohormones such as indole-3-acetic acid[10–12]. Several studies have reported that PGPMs can act synergistically with biochar and liming materials, improving nutrient solubilization, neutralizing soil acidity, and contributing to the regeneration of degraded soils[13,14].

Although numerous studies have examined the individual application of liming agents such as dolomitic lime, biochar, or *Bacillus subtilis*, there is limited experimental evidence on their combined use in acidic soil conditions of the humid tropics. This gap in the literature presents an opportunity to generate new knowledge that can inform the development of integrated technologies for soil rehabilitation and the promotion of sustainable agricultural systems in tropical regions.

In this context, the present study aimed to evaluate the effect of *Bacillus subtilis* inoculation in acidic soil amended with biochar and liming materials on the agronomic performance and crude protein content (for forage purposes) of the hard yellow maize 'Marginal 28 T' variety under humid tropical conditions. The findings are expected to provide valuable scientific evidence to support the design of sustainable and replicable agricultural practices for production systems in the humid tropics.

Methods

Study location

The research was conducted on the experimental plots of the San Ramón Agrarian Experimental Station (EEA) as part of the National Institute of Agrarian Innovation (INIA). The station is located at kilometer 6 of the Yurimaguas-San Ramón Highway in the Yurimaguas district, Alto Amazonas province, Loreto region, Peru. It is situated at 5°56′09" S, 76°07′12" W, at 143 m above sea level.

Field characteristics and environmental conditions of the study

According to the World Reference Base for Soil Resources (WRB) classification system, the study site is classified as an Eutric Fluvisol and an Eutric Gleysol soil type. The physiography is a non-floodable, high-terrace plain, as described by the National Office of Natural Resources Evaluation (ONERN). The field has been used for research on hard yellow maize and rice for over five years. During this period, the site has undergone soil mechanization, crop residue incorporation, and the conventional application of chemical fertilizers and pesticides.

The study area's life zone is classified as a tropical rainforest, following the classification by Aybar-Camacho et al.[15]. It features a warm, rainy climate with abundant precipitation throughout the year. Castro et al.[16] described the region as very humid (B(r)A'H4) across all seasons.

Climatic data were collected from the San Ramón conventional station, operated by the National Service of Meteorology and Hydrology (SENAMHI). The average temperature recorded was 26.9 °C, with a minimum of 21.7 °C and a maximum of 32.1 °C. The accumulated rainfall totaled 398.4 mm, which is below the typical range of 500 to 650 mm as reported by Diaz-Chuquizuta et al.[17]. This indicates prolonged periods without rainfall, as illustrated in Fig. 1.

Following a zigzag pattern, soil sampling was conducted 30 days before sowing in a 1,200.0 m^2 area. Ten sub-samples were collected from the top 20 cm of soil using a 50 cm T-shaped auger. These sub-samples were then homogenized to form a 1.0 kg composite sample, which was sent to the INIA's Soil, Water, and Foliar Laboratory at the EEA El Porvenir. Analyses were performed to determine organic matter using the Walkley–Black method, total nitrogen using the Kjeldahl-N method, phosphorus using the Bray and Kurtz method, exchangeable potassium with 1N ammonium acetate, pH (1:2, soil:water ratio), acidity, and exchangeable aluminum using the potassium chloride method, and cation exchange capacity (CEC) using ammonium acetate at neutral

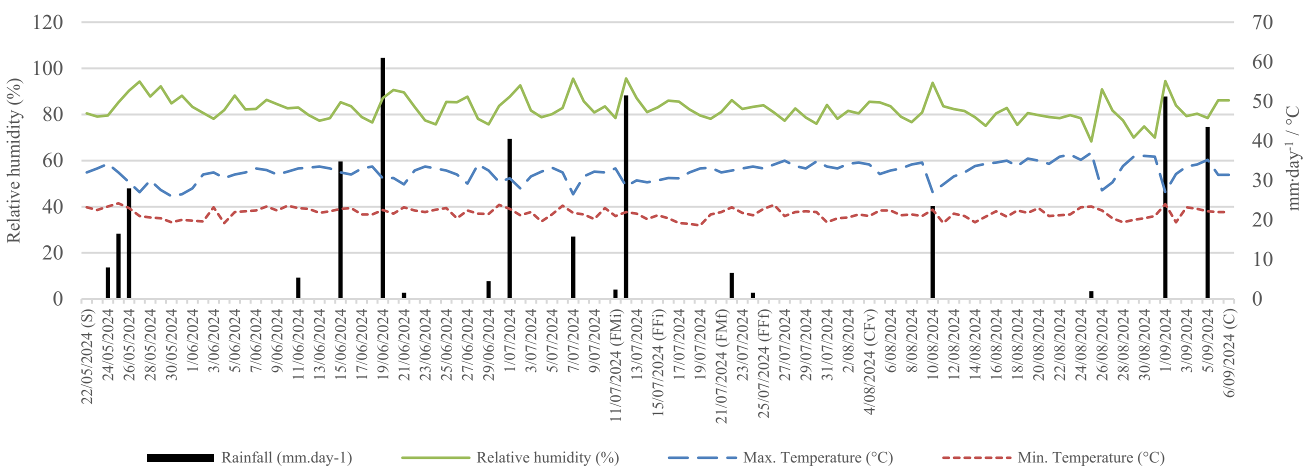

Fig. 1. Climate behavior during this research development. S: Sowing; BMF: Beginning of male flowering; EMF: End of male flowering; BFF: Beginning of female flowering; EFF: End of female flowering; HGF: Harvesting of green forage.

Parameter	Unit	Result
pH	–	4.5
EC	mS·m^{-1}	8
OM	g·kg^{-1}	24
N	g·kg^{-1}	0.9
P	g·kg^{-1}	2.1
K	mg·kg^{-1}	23.19
Ca^{+2}	C mol(+)·kg^{-1}	0.82
Mg^{+2}	C mol(+)·kg^{-1}	0.1
K^{+}	C mol(+)·kg^{-1}	0.06
Na^{+}	C mol(+)·kg^{-1}	0.03
Al^{+3}	C mol(+)·kg^{-1}	0.36
Exchangeable acidity	C mol(+)·kg^{-1}	0.5
CECe	C mol(+)·kg^{-1}	1.51
Sand	%	68.16
Silt	%	16.66
Clay	%	15.18

Table 1. Soil's physical and chemical characteristics of the experimental plot for hard yellow maize Marginal 28 T variety, under rainfed conditions, before the application of amendments. EC: Electrical conductivity; OM: Organic matter; N: Available nitrogen; P: Available phosphorus; K: Available potassium; Ca^{+2}: Exchangeable calcium; Mg^{+2}: Exchangeable magnesium; K^{+}: Exchangeable potassium; Na^{+}: Exchangeable sodium; Al^{+3}: Exchangeable aluminum; CECe: Effective cation exchange capacity.

Parameter	Unit	M0	WOA-WB	WOA-WOB	B-WB	B-WOB	CO-WB	CO-WOB	CC-WB	CC-WOB	D-WB	D-WOB
pH	unit. H	4.50†	4.70†	4.40†	4.90†	4.20†	4.30†	4.10†	5.00†	4.60†	4.60†	4.00†
EC	mS·m^{-1}	8.00*	7.90*	8.50*	6.90*	7.30*	2.50*	10.00*	4.80*	4.90*	6.70*	6.10*
OM	%	2.40↔	1.90↓	1.60↓	1.10↓	1.70↓	1.80↓	1.60↓	1.70↓	1.70↓	1.70↓	1.80↓
N	%	0.09↓	0.10↔	0.08↓	0.06↓	0.09↓	0.09↓	0.08↓	0.09↓	0.09↓	0.09↓	0.09↓
Available P	mg·kg^{-1}	2.10↓	12.30↓	10.50↓	20.10↔	25.90↔	5.00↓	11.70↓	6.40↓	10.50↓	7.80↓	3.40↓
Available K	mg·kg^{-1}	23.19↓	3.20↓	31.20↓	29.20↓	56.80↓	46.40↓	22.40↓	34.80↓	34.39↓	30.39↓	25.99↓
Exchangeable Ca	C mol(+)·kg^{-1}	0.82↓↓	1.79↓↓	1.34↓↓	1.70↓↓	0.96↓↓	1.14↓↓	1.37↓↓	2.43↓	1.59↓↓	1.59↓↓	1.09↓↓
Exchangeable Mg	C mol(+)·kg^{-1}	0.10↓↓	0.28↓↓	0.28↓↓	0.50↓	0.44↓↓	0.27↓↓	0.21↓↓	0.33↓↓	0.35↓↓	0.32↓↓	0.26↓↓
Exchangeable Na	C mol(+)·kg^{-1}	0.03↓↓	0.13↓↓	0.13↓↓	0.14↓↓	0.13↓↓	0.14↓↓	0.13↓↓	0.13↓↓	0.13↓↓	0.15↓↓	0.15↓↓
Exchangeable K	C mol(+)·kg^{-1}	0.06↓↓	0.14↓↓	0.14↓↓	0.26↓	<0.10↓↓	<0.10↓↓	0.10↓↓	<0.10↓↓	<0.10↓↓	0.19↓↓	0.10↓↓
Exchangeable Al	C mol(+)·kg^{-1}	0.36↓	<0.1↓↓	<0.1↓↓	<0.1↓↓	<0.1↓↓	<0.1↓↓	<0.1↓↓	<0.1↓↓	<0.1↓↓	<0.1↓↓	<0.1↓↓
Exchangeable Acidity	C mol(+)·kg^{-1}	0.50↓	0.50↓	0.90↓	0.50↓	1.10↔	1.50↔	1.20↔	0.20↓	0.80↓	0.60↓	1.00↓
CECe	C mol(+)·kg^{-1}	1.51↓↓	2.84↓↓	2.80↓↓	3.05↓↓	2.74↓↓	3.09↓↓	3.05↓↓	3.20↓↓	2.92↓↓	2.82↓↓	2.62↓↓
Texture		SL	SL	SL	SL	SL	SL	SL	SL	SL	SL	SL

Table 2. Comparison of soil chemical and physical properties before and after application of amendments with and without *B. subtilis*. M0: Initial sample; WOA: Without amendment; B: Biochar; CO: Calcium oxide; CC: Calcium carbonate; D: Dolomite; WB: With *B. subtilis*; WOB: Without *B. subtilis*, SL: Sandy loam; *: Normal; ↔: Medium; ↓: Low; ↓↓: Very low; †: Strongly acidic.

pH[18,19]. The results were interpreted based on the procedures outlined by the Mexican standard NOM-021-SEMARNAT-2000 [20], as shown in Table 1.

Changes in soil chemical and physical properties following amendment application with and without *Bacillus subtilis*

Table 2 compares the initial soil properties (M0) with the results obtained after the application of amendments, both with (WB) and without (WOB) *B. subtilis*, revealing significant changes in soil chemical properties. A general increasing trend in pH is observed across treatments, mainly with amendments such as biochar, calcium carbonate, and dolomite when combined with *B. subtilis*. For instance, the addition of calcium carbonate with *B. subtilis* increased the soil pH by 0.50 units, whereas the increase was smaller in its absence (WOB). This highlights the role of *B. subtilis* in neutralizing soil acidity. Conversely, in some treatments without *B. subtilis*, such as biochar, a slight decrease in pH was observed, suggesting a reduced capacity to increase pH without microbial intervention.

Electrical conductivity (EC) showed no consistently significant changes across treatments. In contrast, organic matter (OM) exhibited a slight reduction in all treatments compared to the initial value. While these decreases were minor, they suggest accelerated mineralization—a common phenomenon in tropical environments—that may impact long-term soil fertility if not adequately compensated.

One of the treatment's most notable effects is the significant increase in phosphorus availability. Treated soils with biochar, both with and without *B. subtilis*, showed much more pronounced increases, moving from low to medium phosphorus levels. Other treatments also exhibited favorable increases compared to the initial soil sample, highlighting the crucial role of amendments, whether or not they contained *B. subtilis*, in enhancing phosphorus solubilization in acidic soils.

Regarding available potassium, although the results across all treatments remained low, an increase in $mg \cdot kg^{-1}$ of this element was observed. This suggests a potential synergy between the amendments and *B. subtilis*, contributing to the increased availability of this nutrient.

Finally, exchangeable cations, such as calcium and magnesium, also exhibited significant increases in soils treated with *B. subtilis*. In particular, calcium showed a notable increase in calcium carbonate treatments with *B. subtilis*, highlighting the effectiveness of this treatment in enhancing cation exchange capacity (CEC). Magnesium also improved in the treated soils, although the increase was less pronounced when biochar was combined with *B. subtilis*.

Therefore, amendments applied with *B. subtilis* improve soil pH and the availability of key nutrients such as phosphorus and potassium, enhance organic matter retention, and increase cation exchange capacity. These combined effects suggest that *B. subtilis* incorporation can effectively rehabilitate acidic soils and improve agricultural productivity.

All amendment treatments successfully reduced this value to < 0.10 C mol(+)·kg^{-1}, eliminating toxic soil aluminum effectively. This decrease of at least 0.26 C mol(+)·kg^{-1} is crucial for root growth, as high levels of aluminum are highly detrimental to crops in acidic soils.

Significant differences were observed between treatments (Table 2). For example, biochar without *B. subtilis* (B-WOB) showed an increase of 0.60 C mol(+)·kg^{-1} in exchangeable acidity compared to the initial soil sample (M0), indicating that this treatment was ineffective in neutralizing acidity. In contrast, the calcium carbonate treatment with *B. subtilis* (CC-WB) reduced acidity by 0.30 C mol(+)·kg^{-1} relative to the initial soil value, significantly improving soil quality for maize.

Experimental design

A completely randomized block design (CRBD) with a 2×5 factorial arrangement was employed. The first factor corresponded to the application of the inoculant (with and without *B. subtilis*), while the second factor comprised five treatments: a control (no amendment), biochar (from rice husks), and three types of liming materials (calcium oxide, calcium carbonate, and dolomitic lime), resulting in a total of 10 treatment combinations (Table 3). These were distributed across three blocks. Each experimental unit consisted of six furrows of 6.0 m in length and spaced 0.8 m apart, with a net plot area of 3.20 m^2.

Genetic material

This study utilized hard yellow maize seeds of the Marginal 28 T variety (Registration No. 036-INIPA), which were derived from genetic material developed by the International Maize and Wheat Improvement Center (CIMMYT). This variety is well-adapted to the coastal and rainforest conditions of Peru[17].

Inoculant material

A commercial inoculant based on *Bacillus subtilis* provided by Fungicol Perú SAC, with a concentration of 5×10^8 CFU·mL^{-1}, was used. Applications were carried out according to the manufacturer's recommendations (2 L· ha^{-1}), using a dose of 300 mL per 20 L of water and applied with a manual lever sprayer. Two applications were performed: the first at the V6 vegetative stage (six true leaves), when the plants were less than 50 cm tall,

Treatment code	Applied amendment	Applied dose	*B. subtilis* inoculation	Detailed description
CT-WOB	Without amendment (control)	–	No	Acidic soil without amendment or inoculation
CT-WB	Without amendment (control)	–	Yes	Acidic soil inoculated with *B. subtilis* without amendments
B-WOB	Biochar (rice husk)	25.00 t·ha^{-1}	No	Biochar incorporated 30 days before sowing
B-WB	Biochar (rice husk)	25.00 t·ha^{-1}	Yes	Biochar incorporated 30 days before sowing + inoculation with *B. subtilis* at V6 and V9 stages
CO-WOB	Calcium oxide (Agrocal Mix)	0.81 t·ha^{-1}	No	Incorporated into the soil (0–20 cm) 30 days before sowing
CO-WB	Calcium oxide (Agrocal Mix)	0.81 t·ha^{-1}	Yes	Calcium oxide + *B. subtilis*
CC-WOB	Calcium carbonate (Magnecal)	1.45 t·ha^{-1}	No	Amendment with 77% $CaCO_3$ and 19% $MgCO_3$
CC-WB	Calcium oxide (Magnecal)	1.45 t·ha^{-1}	Yes	Calcium carbonate + *B. subtilis*
D-WOB	Dolomite	1.07 t·ha^{-1}	No	Amendment with 29% CaO and 18% MgO
D-WB	Dolomite	1.07 t·ha^{-1}	Yes	Dolomite + *B. subtilis*

Table 3. Description of treatments under study. CT-WOB / CT-WB: Control treatment with/without *Bacillus* inoculation. B / CO / CC / D: Biochar, Calcium oxide, Calcium oxide, Dolomite. WB / WOB: With or Without *Bacillus* inoculation.

applied both to the soil and leaves; and the second at the V9 stage (nine true leaves), applied directly to the soil. These application stages were selected based on the recommendations of Díaz-Chuquizuta et al.[21] and García-Gonzales et al.[22] for hard yellow maize under rainforest conditions.

Liming material

The liming materials were of commercial origin, while the rice husk biochar was obtained through pyrolysis at over 700 °C and then ground to pass through a No. 10 mesh sieve (2 mm).

Based on the results of the soil analysis, the application doses for each amendment were calculated as follows: 0.81 t·ha^{-1} of calcium oxide, 1.07 t·ha^{-1} of dolomitic lime, 1.45 t·ha^{-1} of calcium carbonate, and 25.00 t·ha^{-1} of biochar. These materials were incorporated into the top 20 cm of soil 30 days before sowing, taking into account the area of each experimental unit (28.8 m^2). The soil was then left to stabilize for 45 days, as recommended by Calva and Espinosa[23]. The composition of each liming material and the biochar is presented in Table 4.

Agricultural activities

The soil was prepared using agricultural machinery fitted with a 250 mm disc plow. Subsequently, the experimental units were delineated. Spray irrigation was applied one day before sowing for a duration of 3 h. On the sowing day (22/05/2024), the seeds were treated with thiodicarb and imidacloprid insecticides at 15 mL·kg^{-1} of seed. After the insecticide was absorbed, two seeds were sown at each planting point. Each furrow contained 21 sowing points spaced 28 cm apart. Supplementary sprinkler irrigation was performed seven days after sowing (DAS), and thinning was conducted at 15 DAS, leaving one plant per sowing point. The study was conducted from May 22 to September 6, 2024.

Based on the soil analysis, nitrogen (urea), phosphate (DAP), and potash (KCl) fertilizers were applied to the crop. The total applied doses were 187 kg·ha^{-1} of urea, 228 kg·ha^{--1} of DAP, and 269 kg·ha^{-1} of KCl. These fertilizers were locally incorporated into the soil, at a 10 cm depth from the plant collar, at two key stages in the crop's development: 40% of the urea and all the phosphorus and potassium were applied at the V3 vegetative stage, while the remaining nitrogen was supplied at the V7 stage.

A preventive application of chlorantraniliprole insecticide was performed at a dose of 10 mL·20 L^{-1}. Weed control was conducted twice using the selective herbicide nicosulfuron at a dose of 50 mL·20 L^{-1}. Due to irregular rainfall during the experiment, four supplementary sprinkler irrigations were carried out during critical growth stages to ensure optimal crop development. These irrigations were applied at the V2 (two true leaves), V10 (ten true leaves), VT (panicle emergence), and R2 (blister grain) stages, following the recommendations of Diaz-Chuquizuta et al.[17].

Assessed variables

The assessed variables and their respective evaluation conditions are detailed in Table 5.

Statistical analysis

The field data collected were organized in Excel. Statistical analyses included the Shapiro–Wilk normality test and Levene's test for homogeneity of variance (homoscedasticity). Analysis of variance (ANOVA), mean

Product	Content
Calcium oxide (Agrocal Mix)	$Ca(OH)_2$: 35–40%
	$CaCO_3$: 1–5%
	MgO: 11–15%
	$CaSO_4$: 30–35%
	SiO_2: 10–15%
Dolomite	CaO: 29%
	MgO: 18%
Calcium carbonate (Magnecal)	$CaCO_3$: 77%
	$MgCO_3$: 19%
Rice husk biochar*	pH: 6.5 Bulk density: 0.127 g·cm^{-3}
	Electrical conductivity: 34.6 mS·m^{-1}
	Organic matter: 46 g·kg^{-1}
	Total nitrogen: 0.38 g·kg^{-1}
	Phosphorus: 500 mg·kg^{-1}
	Potassium: 300 mg·kg^{-1}
	Calcium: 3600 mg·kg^{-1}
	Magnesium: 700 mg·kg^{-1}

Table 4. Chemical content of the liming materials and biochar applied in the experiment. *: Result of soil, water, and foliar laboratory analysis conducted by the National Institute of Agrarian Innovation (INIA), El Porvenir Agrarian Experimental Station (EEA).

Variable	Measurement unit	Measurement instrument	Evaluation frequency	Procedure description/calculation
Male flowering days	days	Calendar	At the beginning of flowering	50% of the plants reach the vegetative stage VT (visible panicle)
Female flowering days	days	Calendar	At the beginning of flowering	50% of the plants reach the reproductive stage R1 (visible stigmas)
Stem diameter	cm	Digital Vernier	40 and 60 DAS	Third internode below the main ear
Leaf length	cm	Flexometer	40 and 60 DAS	From the main ear leaf
Leaf width	cm	Flexometer	40 and 60 DAS	From the main ear leaf
Leaf area	cm^2	Calculation	40 and 60 DAS	Calculation: (Length x Width) × 0.75
Plant height	cm	Flexometer	40 and 60 DAS	The height of 10 plants was measured per experimental unit
Chlorophyll content	SPAD units	SPAD Meter	40 and 60 DAS	Direct meter reading on selected leaves[24]
Green forage weight (GFW)	kg	Scale	75 DAS	Weighing all green forage in the field
Green forage yield (GFY)	t ha^{-1}	Calculation	75 DAS	Calculation according to equation GFY = (10 000 m^2 /A^2) x (GF FW) × 1000 GFY = Green forage yield per hectare (ha) A^2 = Cut area of green forage in m^2 1000 = (kg to t conversion factor) GF FW = green forage field weight (kg)
Cob weight	kg	Scale	108 DAS	Weighing of harvested cobs
Grain yield (GY)	t ha^{-1}	Calculation	108 DAS	Calculation according to equation GY = (10,000 m^2/Area) × FW × SI × Hf)/1000 GY is grain yield in t ha^{-1}; 10 000 is the m^2 of a hectare; Area is the harvested area of the experimental unit (m^2).; FW is the field weight of harvested cobs per plot; SI is the shelling index (grain weight/cob weight); Hf is the humidity correction factor adjusted to 14%, and 1000 is the conversion factor in ha^{-1}
Leaf weight	g	Digital scale	108 DAS	Weighing of the leaves of the selected plants
Stem weight	g	Digital scale	108 DAS	Weighing of the stems of the selected plants
Dry matter yield	t ha^{-1}	Calculation	108 DAS	Calculation from the dry weight of plants
Crude protein percentage	%	Kjeldahl method	108 DAS	Laboratory analysis on dry samples

Table 5. Variables under study, moments, conditions, and formulas for data collection.

comparisons using Tukey's test at a 5% significance level, and Pearson's correlation were conducted using RStudio v. 4.3.1 and the agricolae package[25] to assess relationships between variables.

Results

Effect of *B. subtillis* inoculation in acidic soil amended with biochar and liming materials on the agronomic performance of Marginal 28 T hard yellow maize

The normality and homogeneity of variance tests for the evaluated variables revealed no significant differences, indicating that the data are normally distributed and that the variability between groups is homogeneous. The analysis of variance demonstrated substantial and highly significant effects for most of the assessed variables (Table 6). Notably, the interaction between soil amended with biochar and liming materials and the inoculation with *B. subtilis* indicates a synergistic effect, underscoring the significance of integrating these combined practices.

The variables plant height (PH), ear height (EH), stem diameter (SD), and leaf chlorophyll content (LCC) exhibited highly significant effects ($p < 0.01$) both individually and in the interaction between factors ($p < 0.05$). Similarly, stem diameter (SD), grain yield (GY), green forage yield (GFY), and dry matter (DM) showed highly significant effects ($p < 0.01$), both as independent factors and in interaction with *Bacillus subtilis*. These results suggest that the combination of amendments and *B. subtilis* inoculation has a significant effect on these variables. In contrast, for other variables such as male (MF) and female (FF) flowering, amendments alone were highly significant ($p < 0.01$), indicating that amendments have a more significant impact on flowering timing, potentially shortening the crop's phenological cycle.

Leaf area (LA) exhibited a highly significant effect on the use of amendments and *B. subtilis*. However, the interaction between these factors was insignificant, suggesting that they act independently, with no evidence of synergy or significant interference between them.

Figure 2A illustrates the interaction between amendments (no amendment, biochar, calcium oxide, calcium carbonate, and dolomite) and *Bacillus subtilis* application (WB = with *B. subtilis*, WOB = without *B. subtilis*) over the days to male flowering (MF) and female flowering (FF). Significant differences in flowering time were observed based on the type of amendment and the application of *B. subtilis*. Treatments without amendments exhibited the longest times to flowering, with the highest values for both MF (MSD = 1.94691) and FF (MSD = 1.93562). In contrast, biochar and calcium oxide significantly reduced the days to flowering. Additionally, treatments with *B. subtilis* (WB) further decreased flowering time. The statistical analysis reveals that treatments combining biochar or calcium oxide with *B. subtilis* (MF-WB, FF-WB) resulted in earlier flowering compared to other treatments.

Figure 2B illustrates the interaction between amendments and *B. subtilis* application on plant height (PH) and ear height (EH), revealing significant differences among the applied treatments. Statistical analysis confirms that the interaction between amendments and *B. subtilis* application was highly significant for both variables. Amendments alone significantly affected plant height (MSD = 22.334) and ear height (MSD = 22.304), suggesting

SV	SW	Levene	Liming material and biochar (Lb)	*B. subtilis* (B)	Lb x B	CV	MSE	Mean
			p > value			%		
MF (days)	0.274 ns	0.329 ns	< 0.0001**	0.4363 ns	0.0375*	1.39	0.64	57.7
FF (days)	0.065 ns	0.053 ns	< 0.0001**	0.4337 ns	0.0836 ns	1.34	0.63	59.55
PH (cm)	0.084 ns	0.484 ns	< 0.0001**	0.0008**	0.0257*	4.69	84.32	195.79
EH (cm)	0.118 ns	0.523 ns	< 0.0001**	0.001**	0.0297*	7.93	84.09	115.69
LA (cm^2)	0.173 ns	0.538 ns	< 0.0001**	0.003**	0.1957 ns	6.8	77.17	129.26
SD (mm)	0.417 ns	0.161 ns	< 0.0001**	< 0.0001**	< 0.0001**	2.07	0.24	23.65
LCC ($mg \cdot cm^{-2}$)	0.506 ns	0.109 ns	< 0.0001**	< 0.0001**	0.0332*	3.98	3.3×10^{-6}	0.046
GY ($t\ ha^{-1}$)	0.283 ns	0.233 ns	< 0.0001**	< 0.0001**	< 0.0001**	3.73	0.01	2.81
GFY ($t\ ha^{-1}$)	0.388 ns	0.143 ns	< 0.0001**	0.0073**	< 0.0001**	7.81	2.69	20.99
DMY ($t \cdot ha^{-1}$)	0.385 ns	0.1443 ns	< 0.0001**	0.0072**	< 0.0001**	7.82	0.59	9.98

Table 6. Analysis of variance for *B. subtilis* inoculation in acidic soil amended with biochar and liming materials on the agronomic variables of the hard yellow maize Marginal 28 T variety. SV: Source of variation; SW: Shapiro Wilks normality; Lb x B: interaction between liming material and biochar (Lb) with *B. subtilis* (B) inoculation; CV: Coefficient of variation; MSE: Mean square of error; MF: Male flowering; FF: Female flowering; PH: Plant height; EH: Ear height; LA: Leaf area; SD: Stem diameter; LCC: Leaf chlorophyll content; GY: Grain yield; GFY: Green forage yield; DMY: Dry matter yield. ns: not significant; *statistically significant; **highly statistically significant.

that the improvements in the soil's chemical and physical properties through amendments had a considerable impact on maize growth. Additionally, using *B. subtilis* as a biostimulant demonstrated significant effects, particularly in combination with biochar, which resulted in the highest plant and ear heights.

The interaction between amendments and the application of *B. subtilis* on stem diameter (SD) and leaf area (LA) revealed significant differences among treatments (Fig. 3A). Statistical analysis indicates that amendments and the use of *B. subtilis* have a significant effect on these variables. The interactions between amendments and *B. subtilis* were also highly significant, particularly for the leaf area, where the treatment combining biochar with *B. subtilis* achieved the highest values. For stem diameter, the differences were more subtle; however, treatments with biochar and calcium oxide also stood out.

In Fig. 3B, the interaction between amendments and the application of *B. subtilis* for leaf chlorophyll content (LCC) reveals significant differences between treatments, as determined by Tukey's mean test at a 0.05 significance level (MSD = 0.00443). It is observed that the combination of biochar and inoculation with *B. subtilis* (WB) leads to a significant increase in chlorophyll content compared to the treatment without amendment and other treatments, such as dolomite. Additionally, including *B. subtilis* across various treatments generally enhances LCC, although the extent of the effect varies depending on the type of amendment applied.

Figure 3C displays the interaction between amendments and *B. subtilis* for grain yield (GY) and green forage yield (GFY), demonstrating that amendments significantly influence both parameters. The interaction is also significant, indicating that the response to amendments depends on inoculation with *B. subtilis*. The treatment combining biochar and *B. subtilis* yields the highest grain yield (MSD = 0.25433), while dolomite generally yields lower results than the other treatments. A similar trend is observed for GFY, where combining biochar and *B. subtilis* produces the highest values (MSD = 3.98782), whereas dolomite results in the lowest.

Figure 3D presents dry matter yield (DMY), significantly responding to amendments and inoculation with *B. subtilis*. In the absence of *B. subtilis*, the treatments with calcium oxide show a substantial increase in dry matter yield. In contrast, the other treatments, both with and without amendments, yield the lowest (MSD = 3.08781). This indicates a clear distinction in the behavior of the treatments.

Correlation between morphological, yield, and nutritional characteristics following the application of amendments and *B. subtilis* in hard yellow maize Marginal 28 T Variety

Pearson's correlation analysis (Fig. 4) revealed several significant relationships among growth, yield, and quality variables of hard yellow maize grown under acidic soil conditions. A particularly strong and significant correlation was observed between male flowering (MF) and female flowering (FF) ($r = 0.99$, $p < 0.01$), indicating that these phenological events are closely related, suggesting a natural synchronization in the maize growth cycle induced by the interaction of *B. subtilis* inoculation in soil amended with biochar and liming materials.

Conversely, plant height (PH) and ear height (EH) exhibit negative and highly significant correlations with MF and FF ($r = -0.81$, $p < 0.01$), suggesting that a longer flowering cycle is associated with smaller plant and ear dimensions. In contrast, stem diameter (SD) shows a strong positive correlation with PH ($r = 0.96$, $p < 0.01$), EH ($r = 0.97$, $p < 0.01$), and other variables such as leaf area (LA). This indicates that plants exhibiting more significant vegetative development, as influenced by the application of soil amendments and *B. subtilis*, tend to develop more robust stems, reflecting a stronger and healthier structural framework.

Grain yield (GY) also shows a strong and significant correlation with PH ($r = 0.83$, $p < 0.01$) and LA ($r = 0.95$, $p < 0.01$), highlighting the importance of vegetative development in biomass accumulation and productivity through the application of biochar and liming materials with *B subtilis* inoculation in acidic soil. However,

Fig. 2. Effect of the interactions of amendment use with (WB) and without (WOB) *B. subtilis* application on the variables (**A**) days to male flowering (MF) and female flowering (FF), and (**B**) plant height (PH) and ear height (EH). WOA means without amendments. Means with different lowercase letters indicate significant statistical differences, as determined by Tukey's test at a 0.05 significance level.

green forage yield (GFY) and dry matter yield (DMY) show more moderate correlations, indicating a less direct interaction between these variables and growth characteristics.

Regarding crude protein (CP), Pearson's correlation analysis (Fig. 4) reveals no significant relationships with the primary growth and yield evaluated variables, as the correlation coefficients are low and not statistically significant ($p > 0.05$). The strongest correlation is observed between CP and ear height (EH), but this relationship remains weak and non-significant ($r = 0.21$, $p > 0.05$). These findings suggest that crude protein accumulation in hard yellow maize is not directly associated with vegetative development or plant yield under acidic soil conditions treated with soil amendments and *B. subtilis*.

Discussion

Effect of liming agents and *Bacillus subtilis* on the growth, development, and yield of Marginal 28 T hard yellow maize

The analysis of variance indicates that an integrated strategy combining liming agents and *B. subtilis* is effective in ameliorating acidic soil conditions and enhancing the yield of hard yellow maize. Liming has a positive effect on critical agronomic traits, such as grain yield and plant height[6] by improving soil fertility and increasing the availability of essential nutrients. This effect is particularly notable when biochar is incorporated as a liming agent[5].

The interaction between amendments and *B. subtilis* creates a synergy that reduces flowering time in hard yellow maize[26], enhancing the efficiency of the crop cycle. Combining biochar, calcium oxide, and *B. subtilis* establishes a more favorable soil environment, improving soil structure and nutrient availability, particularly phosphorus and calcium. This promotes more efficient root growth and accelerates phenological development, which can increase grain yield by mitigating abiotic stress[27]. These findings are consistent with previous studies on the use of growth-promoting microorganisms.

The treatment with biochar and *B. subtilis* was the most effective, as it neutralizes soil acidity and enhances the availability of essential nutrients such as calcium and magnesium. *B. subtilis* also promotes growth by fixing nitrogen, solubilizing phosphorus, and producing phytohormones, which explains the increase in plant and cob height[28,29]. This treatment also resulted in the largest leaf area, indicating better nutritional status and photosynthetic capacity, which favored vegetative growth. The stem diameter was also greater with biochar, likely due to improved soil structure and enhanced nutrient retention. Additionally, *B. subtilis* stimulated root development, enhancing nutrient uptake. In the absence of *B. subtilis*, leaf area and stem diameter were smaller, emphasizing the importance of this microorganism in acid soils for improving crop productivity and resilience[30]. Nevertheless, parameters such as Biochar's surface area, porosity, and particle size distribution should have been assessed to elucidate biochar-*Bacillus* interaction mechanisms, representing a methodological limitation in this study.

Leaf chlorophyll content (LCC) is higher when biochar is combined with *B. subtilis* application in hard yellow maize grown in acidic soils, indicating enhanced photosynthetic capacity. This improvement can be attributed to better soil properties, such as increased nutrient retention and reduced aluminum toxicity. *B. subtilis* also optimizes nutrient uptake in the rhizosphere, reinforcing these positive effects[23]. The interaction between amendments and *B. subtilis* highlights the importance of integrated strategies to enhance productivity and sustainability in the acid soils of the Alto Amazonas region.

Using biochar in acidic soils improves green forage yield at 75 days and grain yield at 108 days in hard yellow maize, owing to its ability to enhance water and nutrient retention. The application of *B. subtilis* amplifies these effects by improving nutrient availability and creating a more favorable rhizospheric environment[30]. While dolomite corrects soil acidity, it does not result in the same yield increase as biochar combined with *B. subtilis*.

The dry matter yield at 75 days indicates vegetative growth and biomass accumulation in maize. Applying biochar in combination with *B. subtilis* enhances the availability of essential nutrients, thereby promoting biomass synthesis at this growth stage. Additionally, inoculation with *B. subtilis* alongside dolomite amendment amplifies this effect by increasing nutrient uptake efficiency[28]. In contrast, amendments with calcium oxide and calcium carbonate did not produce significant improvements, highlighting that combining biochar, dolomite, and beneficial microorganisms is the most effective strategy for improving acidic soils for sustainable livestock forage production.

Correlation between morphological, yield, and nutritional characteristics following the application of liming agents and *Bacillus subtilis* in Marginal 28 T hard yellow maize

The synchronization between male and female flowering suggests optimizing this cycle by applying amendments, allowing *B. subtilis* to enhance plant development. However, the negative correlation with plant height indicates that early flowering may reduce light capture, potentially affecting yield. Furthermore, the increased stem diameter and height resulting from applying amendments and *B. subtilis* are positively correlated with grain yield, highlighting the importance of robust structural development in enhancing crop resilience and productivity under adverse conditions[6,31].

The absence of a significant correlation between crude protein and growth or yield variables suggests that grain protein quality is not directly influenced by plant growth-enhancing practices such as liming or biochar. Protein accumulation depends more on factors such as nitrogen management and maize genetics[32,33]. Given its importance for animal feed and forage nutritional value, specific strategies to increase protein content must be implemented for maize-based livestock systems.

The application of amendments with and without *B. subtilis* significantly altered the soil's chemical and physical properties, thereby enhancing its quality and suitability for cultivating hard yellow maize. The pH, initially acidic (4.50), increased notably with the amendments, particularly with biochar and *B. subtilis* (B-WB), reaching 4.90, thereby neutralizing the soil's acidity. Calcium carbonate with *B. subtilis* (CC-WB) also elevated the pH to 5.00, making it a particularly effective option[34].

Electrical conductivity (EC), which reflects salinity, slightly decreased, indicating that the amendments, in combination with *B. subtilis*, improved nutrient uptake. Although organic matter (OM) decreased in all treatments, likely due to increased microbial activity promoting its mineralization, available nitrogen levels did not significantly change[34,35].

One of the most outstanding results was the increase in phosphorus availability, notably when biochar combined with *B. subtilis* (B-WB) was applied, which reached up to 25.90 $mg \cdot kg^{-1}$, promoting maize development in acidic soils. Exchangeable cations (Ca, Mg, Na, K) also increased, enhancing soil fertility, especially with the CC-WB treatment[29].

Overall, the amended soil with biochar and liming materials, combined with inoculation of *B. subtilis*, not only enhanced the availability of essential nutrients but also reduced soil acidity and improved overall soil health. These improvements contributed to increased productivity of hard yellow maize in acidic soils. The findings support the adoption of integrated strategies involving liming agents and biofertilizers to optimize agricultural production under challenging environmental conditions[31,34].

These results are consistent with previous findings by Mosharrof et al.[36] who demonstrated that the combined application of rice husk biochar and dolomitic lime significantly increased soil pH and phosphorus availability (by 137%), contributing to enhanced maize yield by 77.6%. In our study, the treatment combining biochar and *Bacillus subtilis* achieved a phosphorus availability of 25.90 mg·kg^{-1}, likely due to microbial activation and

◀**Fig. 3**. Effect of the interactions of amendment use with (WB) and without (WOB) *B. subtilis* application on the variables (**A**) stem diameter (mm) and leaf area (cm^2); (**B**) leaf chlorophyll content (LCC); (**C**) green forage yield (GFY) and grain yield (GY); and (**D**) dry matter yield (DMY). WOA means without amendments. Means with different lowercase letters indicate significant statistical differences, as determined by Tukey's test at a 0.05 significance level.

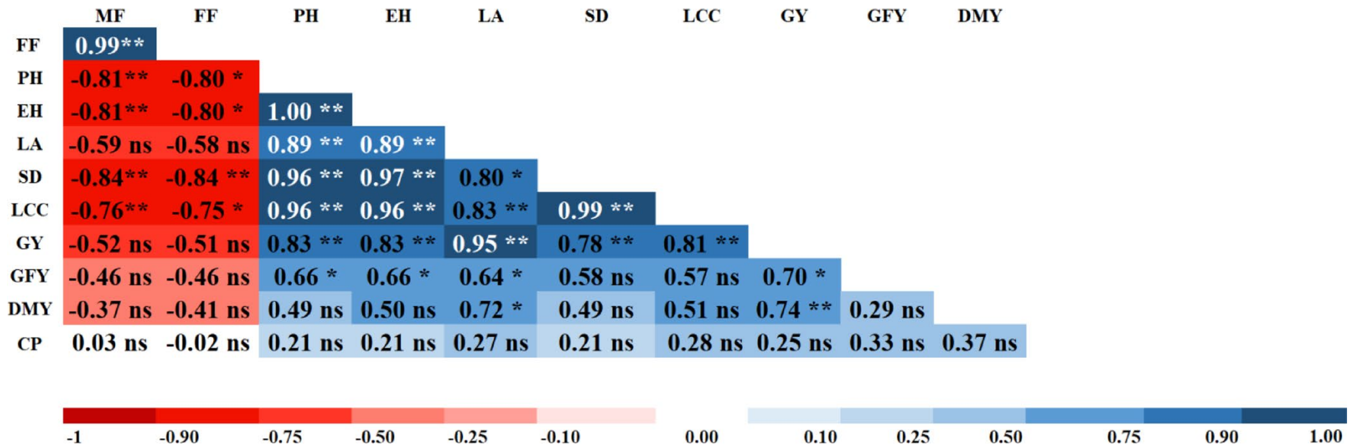

Fig. 4. Pearson Correlation Matrix between morphological characteristics, yield, and crude protein content in yellow maize Marginal 28 T variety, under amendments and *B. subtilis* application. ns: not significant; *statistically significant; **highly statistically significant.

organic acid production, which aligns with the proposed mechanisms of pH buffering and nutrient mobilization by biochar-lime systems.

Conclusions

The amendment of acidic soil with biochar combined with *Bacillus subtilis* inoculation has proven to be a highly effective strategy for enhancing the growth, development, and yield of Marginal 28 T hard yellow maize, both in terms of grain and forage production, under humid tropical conditions. These findings highlight the potential of this approach as an effective strategy for developing sustainable agricultural practices that mitigate soil degradation.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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References

1. Cruz-Macías, W. O. et al. Effect of organic matter and cation exchange capacity on the acidity of soils cultured with corn in two regions of Chiapas, Mexico. *Revista Terra latinoamericana* **38**, 475–480 (2020).
2. Raza, S. et al. Dramatic loss of inorganic carbon by nitrogen-induced soil acidification in Chinese croplands. *Glob. Change Biol.* **26**, 3738–3751 (2020).
3. Maguiña-Maza, R. M. *et al.* Agronomic, productive, nutritional and economic potential of four forage maize genotypes. *Ciencia Tecnología Agropecuaria* **22** (2021).
4. Chávez, A. *et al.* Technologies available to increase corn production in Perú. *ACI Avances en Ciencias e Ingenierías* **14** (2022).
5. Leveau, M., Dumler, S., Anaya De La Rosa, R., Alegre, J. & Ladd, B. Biochar use in the nitrogen balance in aluvial soils of San Ramon/Chanchamayo/Peru. *Ecología Aplicada* **20**, 179–188 (2021).
6. Barandiarán Gamarra, M. Á. & Dirección de Desarrollo Tecnológico Agrario, D. *Manual Técnico Del Cultivo de Maíz Amarillo Duro. Instituto Nacional de Innovación Agraria* (Instituto Nacional de Innovación Agraria, 2020).
7. Pérez-Cabrera, C. A. et al. Rice husk biochar as a substrate for growth of cucumber seedlings. *Rev. Chapingo Ser. Hortic.* **27**, 171–183 (2021).
8. Mondragón-Sánchez, A., Medina-Orozco, L. E., Sánchez-Duque, A. & Núñez-Oregel, V. Effect of the application of biochar in the corn yield in Michoacan, Mexico. *Revista Terra Latinoamericana* **39** (2021).
9. Melo-Lozano, H.-A. & Afanasjeva, N. Effect on microbial activity in soils due to the application of biochar: Review. *Biotecnología en el Sector Agropecuario y Agroindustrial* **21**, 193–209 (2023).
10. Jiang, Z. et al. Application of magnetized ionized water and *Bacillus subtilis* improved saline soil quality and cotton productivity. *Plants* **13**, 2458 (2024).
11. Souza, A. E. S. de *et al.* Application of *Bacillus* spp. Phosphate-solubilizing bacteria improves common bean production compared to conventional fertilization. *Plants* **12**, 3827 (2023).

12. Li, B. *et al.* *Bacillus subtilis* promotes cucumber growth and quality under higher nutrient solution by altering the rhizospheric microbial community. *Plants* **12**, 298 (2023).
13. Rodríguez-Hernández, M. G. *et al.* Native *Bacillus* spp. strains as sustainable alternative in the yield of corn forage. *Revista Terra latinoamericana* **38**, 313–321 (2020).
14. González-León, Y., Ortega-Bernal, J., Anducho-Reyes, M. A. & Mercado-Flores, Y. *Bacillus subtilis* and *Trichoderma*: General characteristics and their agricultural applications. *TIP Revista Especializada en Ciencias Químico-Biológicas* **25** (2022).
15. De, A., De Vida, Z., Perú, D. & Explicativa, G. Atlas de zonas de vida del Perú : guía explicativa. Nota Técnica No 003 SENAMH-DHI-2017. *Repositorio Institucional—SENAMHI* (2017).
16. Castro, A. *et al.* Climas del Perú: mapa de clasificación climática nacional. *Repositorio Institucional—SENAMHI* (2021).
17. Diaz Chuquizuta, P., Hidalgo Meléndez, E., Mendoza Paredes, M. & Jara Calvo, T. W. *Guía Técnica Para El Manejo Del Cultivo de Maíz Amarrillo Duro En La Selva. Instituto Nacional de Innovación Agraria* (Instituto Nacional de Innovación Agraria, 2022).
18. Condori-Ataupillco, T. et al. Biochar-amended soils: A water-saving strategy for quinoa cultivation in the Andes. *J. Sustain. Agric. Environ.* **4**, e70036 (2025).
19. Bazán Tapia, R. Manual de procedimientos de los análisis de suelos y agua con fines de riego. Preprint at https://repositorio.inia.gob.pe/handle/20.500.12955/504 (2017).
20. DOF—Diario Oficial de la Federación. https://www.dof.gob.mx/nota_detalle.php?codigo=717582&fecha=31/12/2002#gsc.tab=0.
21. Díaz-Chuquizuta, P. et al. New thilinear hybrid of hard yellow corn for the Peruvian tropic. *Agronomía Mesoamericana* **34**, 51177–51177 (2023).
22. García-Gonzales, E., Diaz-Chuquizuta, P., Hidalgo-Meléndez, E. & Gil, O. J. A. Response of corn crop to concentrations of digested bovine manure in humid tropical climate. *Manglar* **17**, 203–208 (2020).
23. Calva, C. & Espinosa, J. Effect of four liming materials on acidity control of a soil from Loreto. *Orellana. Siembra* **4**, 110–120 (2017).
24. Conversions. https://www.atleaf.com/SPAD#SPAD.
25. Mendiburu, F. de. agricolae: Statistical Procedures for Agricultural Research. Preprint at https://cran.r-project.org/web/packages/agricolae/index.html (2023).
26. Díaz-Chuquizuta, P., Hidalgo-Melendez, E., Cabrejo-Sánchez, C. & Valdés-Rodríguez, O. A. Response of maize (*Zea mays* L.) to foliar application of liquid organic fertilizers. *Chilean J. Agric. Anim. Sci.* **38**, 144–153 (2022).
27. Iglesias, S., Alegre, J., Salas, C. & Egüez, J. Corn yield (*Zea mays* L.) improves with the use of eucalyptus biochar. *Scientia Agropecuaria* **9**, 25–32 (2018).
28. Ruelas-Islas, J. D. R. *et al.* Phosphorus and *Bacillus subtilis* in absorption and removal of micronutrients in *Phaseolus Vulgaris* L. *Rev Mex De Cienc Agric* 243–252 (2022) https://doi.org/10.29312/remexca.v13i28.3279.
29. Anguiano Cabello, J. C., Olalde Portuga, V., Arredondo Valdés, R. & Laredo Alcalá E. I. Evaluation of *Bacillus subtilis* as promoters of plant growth. *Revista Bio Ciencias* **6** (2019).
30. García-de la Paz, N. C. *et al.* Potential of *Bacillus* native to the Comarca Lagunera as a biofertilizer in the production of forage corn. *Rev Mex De Cienc Agric* **13**, 253–261 (2022).
31. Escalona Sánchez, A., Gavilanez Buñay, T., Francisco Yépez, A. & Ramírez Guerrero, H. O. Use of soil amendments in the production of corn for silage with organic mineral irrigation. *Agronomía Costarricense* 177–192 (2021) https://doi.org/10.15517/rac.v45i1.45769.
32. Chan-Chan, M., Moguel-Ordóñez, Y., Gallegos-Tintoré, S., Chel-Guerrero, L. & Betancur, D. Chemical and nutritional characterization of high quality protein maize (*Zea mays* L.) varieties developed in Yucatan, México. *Biotecnia* **23**, 11–21(2021).
33. Obando, M., Alvarado, A., Hasang, E., Farah, S. & Palacios, C. Yield and protein content evaluation of seven Ecuadorian hybrid corn (Zea mays L.) grown in the Santa Catalina Valley, Peru. *Manglar* **19**, 227–232 (2022).
34. Arianis Santos, A. & Samudio, A. Evaluation of the effect of the inoculation of *Bacillus subtilis* on pH, exchangeable aluminum and availability of phosphorus, in an ultisol. *Investigaciones agropecuarias* **2**, 15–30 (2020).
35. Acurio Vásconez, R. D. *et al.* Evaluation of *Bacillus* spp. as plant growth-promoting rhizobacteria (PGPR) in broccoli (*Brassica oleracea* var. *italica*) and lettuce (*Lactuca sativa*). *Ciencia y Tecnología Agropecuaria* **21**, 1–16 (2020).
36. Mosharrof, M. et al. Combined application of biochar and lime increases maize yield and accelerates carbon loss from an acidic soil. *Agronomy* **11**, 1313 (2021).

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Declarations

Competing interests
The authors declare no competing interests.

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