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Contrasting Effect of Different Organic Amendment Fertilization in Maize and Soil under Different Watering Conditions

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Abstract: Organic amendments are increasingly promoted as sustainable fertilization strategies because of their potential to improve soil fertility and crop productivity. However, their heterogeneous composition can produce contrasting effects on soil properties, microbial communities, and plant performance, particularly under water-limited conditions. This study evaluated the effects of two organic amendments, chicken manure (CHI) and anaerobically digested sludge (SL), on maize (*Zea mays* L.) grown under well-watered (WW) and water-stress (WS) conditions in a greenhouse experiment. Plant physiological responses, soil physicochemical properties, and soil microbial communities were assessed to determine how amendment type influences crop performance under contrasting water regimes. Both amendments modified soil chemical properties, microbial community composition, and maize physiological performance relative to the control, demonstrating that amendment type strongly influences plant–soil interactions. However, their effects differed markedly. SL increased photosynthetic performance, and biomass production under WW conditions. Under WS conditions, SL-treated plants showed pronounced reductions in growth, photosynthesis, stomatal conductance, and chlorophyll fluorescence, accompanied by increased abscisic acid accumulation. In contrast, CHI consistently reduced plant biomass regardless of watering regime and was associated with higher soil electrical conductivity, chloride concentration, and foliar sodium accumulation. Microbial analyses revealed amendment-specific responses, with SL promoting copiotrophic bacterial groups linked to nutrient cycling, whereas CHI reduced the abundance of several bacterial taxa and arbuscular mycorrhizal fungi (Glomeromycota). Water stress had comparatively limited effects on overall microbial community structure. These findings demonstrate that the effects of organic amendments on maize depend not only on nutrient supply but also on amendment-induced changes in soil properties and microbial communities. Amendment composition should therefore be carefully considered when designing sustainable fertilization strategies under variable water availability.

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Keywords: organic amendment; drought; maize; soil microbiota; sustainable fertilization; plant stress

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1. Introduction

Changing environmental conditions and growing global population are relevant challenges submitted in our society. Chemical fertilizers have played a fundamental role in increasing agricultural productivity and ensuring food security by supplying essential nutrients to crops (Zhang & Zhang, 2007). However, their excessive and prolonged application may negatively affect soil quality and nutrient use efficiency, highlighting the need for complementary management strategies that maintain productivity while promoting soil health and sustainability (Bai et al., 2020; Havlin et al., 2023; Saeid & Chojnacka, 2019).

Maize (*Zea mays* L.) is among the most widely cultivated cereal crops globally and is particularly vulnerable to water deficits due to its high water and nutrient requirements. Drought commonly reduces photosynthetic performance, carbon assimilation and biomass accumulation, leading to significant yield losses. Consequently, identifying fertilization strategies capable of sustaining plant physiological performance under limiting water conditions has become a major challenge for sustainable agriculture (Farooq et al., 2015).

Organic amendments understood as compost and biochar compounds are sustainable fertilization managements widely applied in sustainable agriculture nowadays. Those compounds are essential in enhancing soil quality by increasing different relevant parameters as soil organic matter, crop soil structure and aggregate formation (Matisic et al., 2024). This management, has been applied mainly to not only increase the crop productivity, but also ameliorate the impact of different abiotic stresses such as drought, salinity and heavy metals (Irin & Hasanuzzaman, 2024; Morales-Salmerón et al., 2024). Many advantages by using organic amendments have been also observed in different studies by promoting beneficial soil microbiome activities, mineral solubilization, nutrient availability, crop quality and pathogenic resistance. (Akanmu et al., 2021; Azarbad, 2022; Li et al., 2021; Zhang et al., 2023). On the other hand, according to previous studies, organic amendment drive sifts microbial communities taxa promoting bacteria groups well adapted to carbon rich environments -named copiotrophic- while decreasing bacteria groups better adapted to poor environments oligotrophic (Cui et al., 2023; Tang et al., 2020). While organic amendments can improve soil fertility, much less is known about the mechanisms by which specific amendment influence plant drought limitations through changes in soil physicochemical properties and microbial community assembly. Furthermore, the heterogeneous physicochemical composition of organic amendments can induce contrasting effects on soil microbial communities, nutrient dynamics, and plant physiological responses more specifically in maize, making their effectiveness under water-stress conditions highly context-dependent and difficult to predict, mainly due to their soil nutrition dependance and elevated water necessities (Shah et al., 2023).

One of the major challenges associated with soil amendments is predicting how they will interact with abiotic stress factors, particularly drought conditions. By contributing to the improvement of soil structure, increasing water retention capabilities, and stimulating microbial activity, these organic amendments promise to significantly enhance crop productivity (Shi et al., 2024). Nonetheless, under prolonged drought conditions, microbial-mediated decomposition slows down, potentially delaying the release of essential nutrients, which are crucial for plant growth (Canarini et al., 2021). The variability in soil texture, mineral composition, and pre-existing microbial communities further complicates the prediction of amendment efficacy under drought conditions (Lehmann et al., 2011). Consequently, assessing the effectiveness of organic amendments under different soil conditions is essential to better understand their role in enhancing plant tolerance to water stress and improving soil resilience. The communities of microorganisms that inhabit the soil, particularly those groups of copiotrophic bacteria and arbuscular mycorrhizal fungi (AMF), are crucial players in the processes of nutrient mobilization and enhancing plant resilience against various stress factors (Lehmann et al., 2020).

The heterogeneous composition of organic amendments, driven by differences in feedstock sources and processing methods, can produce contrasting effects on soil properties, microbial communities, and crop performance. As a result, predicting their agronomic and ecological outcomes remains challenging. Therefore, the objective of this study was to evaluate how two contrasting organic amendments influence maize performance under well-watered and drought conditions by simultaneously assessing plant physiological responses, soil physicochemical properties, and soil microbial communities. A sludge-based amendment and manure-based amendment were selected because they represent contrasting organic resources commonly used in agricultural systems. Sewage sludge is typically characterized by a relatively high nutrient content and rapid nutrient release, whereas manure contains more stabilized organic matter and is generally associated with gradual improvements in soil structure and water-holding capacity. These contrasting properties make them suitable models for evaluating how amendment composition influences plant, soil, and microbial responses to drought stress.

We hypothesized that organic amendments modify maize responses to drought by altering soil physicochemical properties and microbial community composition, thereby affecting nutrient availability, plant growth, and physiological stress responses. Because amendment composition varies substantially among products.

Therefore, the objective of this study was to evaluate the contrasting effects of two compositionally distinct organic amendments on maize under well-watered and water-stress conditions and to determine how amendment-induced changes in soil are associated with shifts in maize physiology and bacterial and fungal communities.

2. Materials and Methods

2.1. Plant growth and experimental design

The experiment was carried out in the Agrobiotechnology institute (IdAB) regulated greenhouse. Maize seeds (*Zea mays* L.) were germinated in 7.5 L pots (1 plant per pot) containing medium clay soil from UPNA experimental fields in Pamplona (Navarra). The experiment employed a silty clay soil with a pH of 8.2, organic matter at 2.7%, total nitrogen at 1.6%, available phosphorus at 68 ppm, available potassium at 285 ppm, and a cation exchange capacity of 13.3 meq 100 g^{-1}. Before treatment application, fertilization was tailored to meet the crop's needs with a dose of 5 g per pot with (9:18:24) (N:P:K) in all the plants. Half plants identified as well watered plants (WW) were irrigated at pot capacity ensuring they were consistently kept at maximum substrate water-holding capacity (4 replicates per group). The other half identified as water stressed plants (WS) were irrigated at 50% pot capacity after plant were successfully germinated. In the experiment plants were grouped in three different fertilization treatments. Control group, where plants were growth under normal conditions with no amendment application. Sludge waste amendment group (SL) where plants were growth under organic amendment application HKB -HUMIKOIA (F0004835/2031) which composition is shown in Table S1 and third groups identified as chicken manure (CHI) where plants were treated with HCBC1 -HUMICOFERT (F0004143/2029) which composition is shown in Table S2 (4 replicates per treatment). The doses of those organic amendments were the same for both treatments (15 tons ha^{-1}). This dose was selected because it corresponds to the manufacturer's recommended field application rate. The whole experiment was set with natural sunlight conditions, with temperatures maintained at 22/16 °C (day/night) by the air conditioning system of the greenhouse.

When plants reached stage 6 leaves (45 days after germination) applying leaf collar method (Abendroth et al., 2011), analyses of leaf gas exchange, chlorophyll fluorescence (described below) and leaf sampling was performed. Leaf samples were quickly frozen in liquid nitrogen and stored at −80 °C for subsequent analyses. Left plant samples underwent oven drying for 48 h at 60 °C for biomass and mineral composition analyses. At this stage, above ground biomass (leaves and shoots) were analyzed. After harvesting, leaves and shoots were dried at 60 °C in an oven for 48 h to obtain the dry mass of each plant.

2.2. Gas Exchange, pigments and chlorophyll fluorescence determination

The youngest full developed leaf was fully expanded and placed within a portable photosynthesis system, the Li-Cor 6400 (Li-COR, United States), for gas exchange analysis. The rate of CO_2 assimilation under light saturation, denoted as Asat, was determined at a photosynthetic photon flux density (PPFD) of 1200 µmol m^{-2} s^{-1} using established equations by von Caemmerer & Farquhar, (1981). Plant water status was assessed by measuring stomatal conductance to water vapor (g_s) following the methodology described by Harley et al. (1992). Maximal quantum efficiency of PSII (Fv/Fm) and the relative quantum efficiency of PSII photochemistry (ΦPSII) were simultaneously determined with a fluorescence chamber (LFC 6400-40; Li-COR) connected to the Li-Cor 6400XT portable photosynthesis system. Chlorophyll (Chl), flavonoids (Flav) and anthocyanins (Anth) contents were estimated using a portable non-destructive DUALEX sensor (Dualex Scientific, Force A, Orsay, France) with five measurements performed on the same leaves used for gas exchange analyses. All measurements were collected immediately before harvest for biomass determination and physiological analyses.

2.3. Mineral composition of leaves and soil

100 mg of milled dried maize leaf and soil samples were collected. Micro- and macronutrients were quantified using ICP/OES (inductively coupled plasma/optical emission spectrome-try, iCAP 6500 Duo, Thermo Fisher Scientific, Waltham, MA, USA). Leaf N concentration was obtained by utilizing sample dynamic combustion with an elemental analyzer (FlashEA1112, ThermoFinnigan, Waltham, MA, USA) equipped with a MAS200R autosampler.

2.4. ABA determination

About 40 mg of lyophilized leaf sample were suspended in 80% methanol-1% acetic acid containing internal standards and mixed by shaking for 1 h at 4 °C. The extract was kept at −20 °C overnight and then centrifuged and the supernatant dried in a vacuum evaporator. The dry residue was dissolved in 1% acetic acid and passed through an Oasis HLB (reverse phase) column (Seo et al., 2011). For abscisic acid (ABA) quantification, the dried eluate was dissolved in 5% acetonitrile-1% acetic acid, and the hormone was separated using an autosampler and reverse phase UHPLC chromatography (2.6 µm Accucore RP-MS column, 100 mm length × 2.1 mm i.d.; Thermo Fisher Scientific, Waltham, MA, USA) with a 5 to 50% acetonitrile gradient containing 0.05% acetic acid, at 400 µL/min over 21 min.

2.5. Leaf sugars and amino acids content

Amino acids were determined in 50 mg of frozen fresh matter of flag leaves, (previously ground in a mortar with liquid nitrogen) after derivatization with an ACCQ-Fluor™ Reagent kit (Waters, MA, USA) based in borate buffer, acetonitrile and AQC derivatizing reagent (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) using HPLC system (Thermo Fisher Scientific). Lyophilized flag leaf tissue (25 mg) was homogenized in 0.5 mL of 100% ethanol, then another 0.5 mL of 80% ethanol was added and mixed using a vortex. The sample was incubated at 70 °C for 90 min, centrifuged at 14,000× *g* for 10 min and the supernatant collected. The supernatant was used to determine glucose, fructose with an ionic chromatographer (ICS-3000, Thermo Scientific™, Waltham, MA, USA). Remained pellet was used for Starch measurement adding KOH (0.2 N) and adjusting pH to 4.8 with acetic acid (0.1 N). Kit containing the enzyme amyloglucosidase (R-Biopharm, Darmstadt, Germany) was used for extraction and the absorbance was measured with a spectrophotometer at 340 nm.

2.6. Soil characterization

PP Systems Environmental Gas Monitor (EGM-4) was used to measure CO_2 efflux from soil before harvesting. Soil for metagenomic analyses was collected by using an iron soil extractor at 2 cm deep of the surface and surrounding the plant roots filling a 2 mL Eppendorf tube. Afterwards, full Eppendorf were put in boxes and quickly frozen in liquid nitrogen. Afterward, soil samples were maintained at −80 °C fridges until analyses. Fresh soil was used to determine mineral nitrogen, organic matter content, phosphorus, electrical conductivity and chlorides concentration. Organic matter in soil was determined using Walkley-Black method as described in Poudel (2020). Soil phosphorus was determined using Olsen-Watanabe method as described in De Silva et al., (2015). Electrical conductivity was determined as the level of salts in the aqueous extract, 1:1 (*w*/*v*) ratio of soil in water. Aqueous extract was also used for chlorides determination using potentiometric measurement with Silver Nitrate. For mineral nitrogen concentration in soil a wet extraction with 1N KCl as the extractant, filtration, and spectrophotometric determination of separate measurements at 640 nm for ammonium (colorimetry in the visible spectrum) and at 220 nm (ultraviolet spectrum) for nitrates, considering the absorbance at 275 nm to eliminate potential interference from the soil's organic matter.

2.7. Bioinformatic analyses of 16S rRNA and ITS2 amplicon sequencing data

The composition and structure of the soil sampled bacterial communities was assessed through the amplification and sequencing of the V3–V4 variable regions of the 16S rRNA gene, and the sampled fungal communities were assessed through the amplification and sequencing the internal transcribed spacer 2 (ITS2). The Illumina Miseq sequencing 300 × 2 approach was used for both communities. Amplification was performed after 25 PCR cycles. A negative control of the DNA extraction was included as well as a positive Mock Community control to ensure quality control. Bioinformatics processing and analysis Raw demultiplexed forward and reverse reads were processed using QIIMCHI (Bolyen et al., 2019). Step Methods for bioinformatic analyses were performed according to Dada2 (Callahan et al., 2016). Phylogeny assessment was performed according Mafft (Katoh & Standley, 2013) and Fasttree method (Price et al., 2009). Phylotype data was used to calculate Observed OTUs (community richness). Taxonomic assignment of phylotypes was performed using a Bayesian Classifier trained with Silva database version 138 (99% OTUs full-length sequences) (Wang et al., 2007).

2.8. Statistical analysis

Effect of Both amendment fertilizers (SL and CHI) and watering conditions (WW and WS) and their interaction were statistically analyzed by two-way ANOVA (SPSS v.22; SPSS Inc., Chicago, IL, USA). Differences between treatment groups were tested as significant when p-value < 0.05. Post hoc Tukey. To reduce complexity of the multivariate data and identify patterns, analyzed parameters within each treatment were aggregated and utilized as index values for conducting a Principal Component Analysis (PCA). Abundance of bacterial phyla population were employed to ascertain the correlation between response variable vectors and to make comparisons across the ordination space. Studied parameters were also subjected to heatmap clustering analysis (HCA). k-Means clustering analysis was used to determine the response of studied parameters with the different amendment treatments and watering conditions. This analysis groups together comparable responses, enabling the identification of unique clusters. The Pearson correlation coefficient was employed as a distance metric based on correlation. Afterward, Heatmap was generated using Perseus software. The dissimilarity matrix was computed using the Euclidean technique. PCA biplot analyses were conducted using the RStudio software (Version 2024.09.0+375), employing the 'prcomp' function from the 'factoextra' package (Kassambara & Mundt, 2020).

3. Results

3.1. Plant growth, Gas exchange and chlorophyll fluorescence

Under well-watered (WW) conditions, the sludge-based amendment (SL) significantly increased about (150%) the biomass, stomatal conductance (gs), net CO_2 assimilation, PSII operating efficiency (ΦPSII), and electron transport rate (ETR), while moderately increasing PSII quantum yield (Fv'/Fm') compared with the control (Figure 1). The relatively low photosynthetic activity observed in control plants under WW conditions suggests the presence of nutrient or soil-related limitations. Under water-stress (WS) conditions, however, SL-treated plants

exhibited a significant reduction in all growth and photosynthetic parameters compared with their well-watered counterparts (≈50% decrease). In contrast, the chicken manure amendment (CHI) consistently reduced plant biomass relative to the control under both watering regimes. Despite this reduction in growth, CHI had limited effects on other physiological parameters. Under WW conditions, net CO_2 assimilation, stomatal conductance, Fv'/Fm', and ΦPSII did not differ significantly from the control. Under WS conditions, CHI-treated plants showed significantly higher net CO_2 assimilation and electron transport rate than the control, whereas stomatal conductance, Fv'/Fm', and ΦPSII remained unchanged between watering treatments.

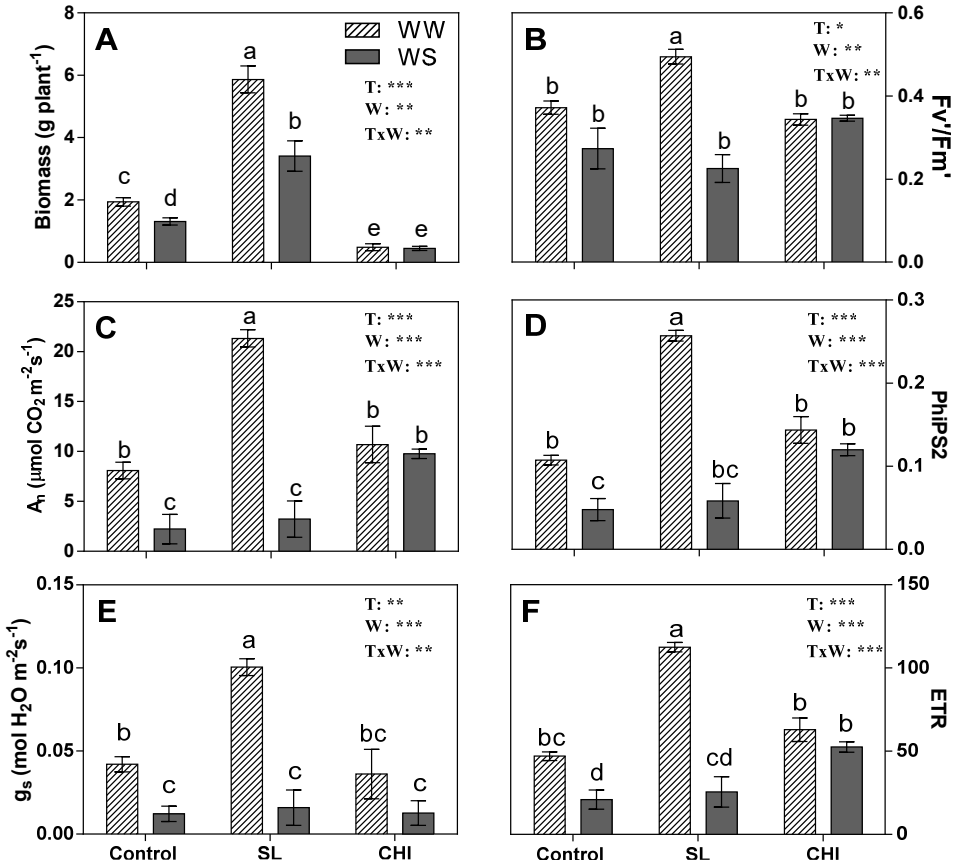

Figure 1. Dry weight biomass (**A**), net [CO_2] assimilation An (**B**), stomatal leaf conductance gs (**C**), efficiency quantum yield of PSII (**D**), PSII efficiency (**E**) and electron transport rate (**F**) of maize plants growth with no treatment (Control), sludge waste-based amendment (SL) and chicken manure amendment (CHI). Well-watered conditions are showed in yellow bars (WW) and water stress conditions (WS) in red bars. Bars are means (n = 4) and capped lines are standard errors. Two-way ANOVA significant differences of Fertilization factor (T), watering factor (W) and interaction (TxW) are represented in each graph with * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$). Within each graph, bars with the different letter indicate that values are significantly different ($p < 0.05$) Post-hoc Tukey.

SL and CHI showed different effects in the accumulation in leaves of different compounds related to stress factors, pigments and carbohydrates. Plants treated with SL amendment showed doubled levels in fructose accumulation and chlorophyll pigments compared to control plants under WW conditions. On the other hand, SL treated plants under WS conditions starch levels were almost depleted, while hormone ABA increased by 2 orders of magnitude compared to plants under WW conditions. CHI amendment decreased soluble sugars (Glucose and Fructose) in both watering conditions by half. Additionally, anthocyanin and flavonoid pigments also decreased significantly in those plants compared to control plants. CHI amendment showed less accumulation of ABA compared to other treatments even under WS conditions compared to control and SL treatments. SL amended plants showed higher concentration of soluble sugars and chlorophyll and lower level of flavonoids compared to CHI amended plants, but SL treatment showed the highest accumulation of ABA under WS conditions. According to these results an interaction between fertilization treatment and watering conditions was found in ABA, Glucose and Chlorophyll content like the previous analyzed parameters (Table 1). Mineral micro- and macronutrients content in leaves tended to increase generally in SL and CHI treatment compared control plants, but the accumulation of certain elements such as magnesium (Mg), manganese (Mn), sodium (Na) and Phosphorus (P) was significantly higher in CHI treatment than SL treatment (Table S3) consistent with the mineral composition of the applied amendments (Table S4).

Table 1. Concentration of hormone ABA, starch, Glucose, Fructose and leaf pigments (Chl, Anth, Flav) in leaf of maize plants of control group, SL treatment, CHI treatment under two watering conditions (WW and WS) Values are means (n = 4) ± S.E. Within treatments, means for each parameter followed by different letter are significantly different Pos Hoc Tukey ($p \leq 0.05$). Within treatments, means for each parameter followed by different letter are significantly different. ANOVA: ns not significant; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

Treatment	Water	ABA (pmoles g^{-1})	Starch (µmol g^{-1} DW)	Glucose (µmol g^{-1})	Fructose (µmol g^{-1})	Chl (dualex units)	Anth (dualex units)	Flav (dualex units)
Control	WW	102 ± 16d	79 ± 11a	140 ± 16a	143 ± 13b	13.7 ± 0.8c	0.17 ± 0.01a	1.24 ± 0.03a
	WS	5422 ± 1425b	24 ± 11b	186 ± 12a	230 ± 16a	16.2 ± 1.6bc	0.18 ± 0.01a	1.28 ± 0.02a
SL	WW	136 ± 10d	73 ± 6a	207 ± 40a	238 ± 31a	28.4 ± 0.92a	0.12 ± 0.01b	0.68 ± 0.06c
	WS	12013 ± 2484a	8 ± 2c	129 ± 20a	242 ± 18a	26.9 ± 2.2a	0.13 ± 0.01b	0.59 ± 0.11c
CHI	WW	108 ± 31d	54 ± 8ab	56 ± 12c	63 ± 15c	16.5 ± 2.2bc	0.14 ± 0.01b	1.07 ± 0.06b
	WS	1526 ± 138c	30 ± 6b	78 ± 8c	117 ± 12b	20.1 ± 0.8ab	0.15 ± 0.01b	1.08 ± 0.0b
ANOVA	T	**	ns	***	**	***	***	***
	W	***	***	ns	***	ns	ns	ns
	TxW	**	ns	*	ns	*	ns	ns

In this study watering conditions highly affected the accumulation of aminoacids in leaf of maize plants, depending on different applied amendment treatments. No significant accumulation of aminoacids were found between watering conditions (WW & WS) in control plants. SL showed a significant accumulation of asparagine (Asp) glutamine (Glu) and alanine (Ala) compared to control plants under WW optimal conditions. On the other hand, under WS conditions, SL amended plants exacerbate more significant accumulation of asparagine (Asp), glutamine (Glu), serine (Ser), glycine (Gly), alanine (Ala), arginine (Arg), tyrosine (Tyr), valine (Val), isoleucine (Ile), leucine (Leu), phenylalanine (Phe) and total amino acids, compared to control plants (Table S5). As a result, SL treatment under WS conditions supposed a significant accumulation of isoleucine (Kebrom et al., 2019) (Ile), leucine (Leu) and phenylalanine (Phe) compared to SL treated plants under WW conditions. On the other hand, CHI also showed accumulation of different amino acids such as asparagine (Asp) and glutamine (Glu) under WW conditions. Additionally, differences in GABA and tyrosine (Tyr) accumulation between SL and CHI treated plants under WS conditions were obtained. No difference in methionine (Met) was found in any of the treatments and watering conditions (Table S5).

3.2. Soil chemical characterization

Different studied soil parameters changed with different amendment applications. Both SL and CHI increased significantly organic matter and nitrogen concentration by approximately 30% in soil, while watering conditions did not show any significant effect in those parameters (Figure 2A,B). Phosphorus concentration also increased significantly in both SL and CHI treatments but only differences between SL and CHI treatments were found under well-irrigated conditions (Figure 2C). Soil respiration only significantly increased under SL treatment in both irrigation conditions (Figure 2D). Soil electrical conductivity increased in SL treatment and even more in CHI treatment (about 200%). Water stress conditions also increased soil electrical conductivity in SL treatment and even more in CHI treatment (Figure 2E). Ion chlorine concentration showed a similar behaviour to electrical conductivity analysis with also similar effects (Figure 2F).

3.3. Soil bacteria and fungi phyla analysis

Of the 15 analyzed bacterial phyla, 11 different phyla were significantly affected by organic amendment application, while no significative effects in water conditions and their interaction were found (Table 2). The general tendency showed that organic amendment fertilization negatively affected the relative abundance of different bacteria groups such as Planctomycetota, Crenarchaeota, Acidobacteria, Methylomirabilota, Actinobacteriota and Gemmatimonadota mainly under CHI treatment compared to control group. Organic amendments notably diminished the prevalence of Planctomycetota, Crenarchaeota, Acidobacteria, Methylomirabilota, Actinobacteriota, and Gemmatimonadota under CHI relative to the control. In contrast, Proteobacteria, Bacteroidota, and Firmicutes exhibited increased abundance with both treatments. Besides, Actinobacteriota and Gemmatimonadota were significantly reduced in CHI across both conditions.

To better understand the bacterial community's dynamics under different organic amendment treatments and to identify relevant effects in plant-microorganism relationships and soil quality, a heatmap and hierarchical clustering analysis of affected and relevant bacterial groups was performed (Figure 3A). Analyzed bacterial phyla were also identified and grouped depending to its nutritional strategy (Oligotrophs or Copiotrophs) according to (Ho et al., 2017; Stone et al., 2023). The hierarchical clustering of the 6 combinations (two conditions of water and three treatments) formed 2 clusters separating mainly control and CHI groups under WW conditions, while samples of SL treatment could be found in both clusters. On the other hand, hierarchical clusters of the 13 studied phyla were also grouped into two

major clusters. The major clusters (I) located in the left part of the heatmap show the bacterial phyla, which relative abundance tended to increase under amendment applications, while the major cluster located in the right (II) tended to decrease. Copiotrophs bacteria marked in red and Oligotrophic bacteria marked in blue would be expected to be separated in I cluster and II cluster respectively, but there exist 2 exceptions associated with copiotrophic groups: Actinobacteriota and Gemmatimonadota phyla. Histogram of their relative abundance and statistical analysis (ANOVA) showed a significant depletion in CHI treatment under both watering conditions of Actinobacteriota (Figure 3B) and Gemmatimonadota (Figure 3C).

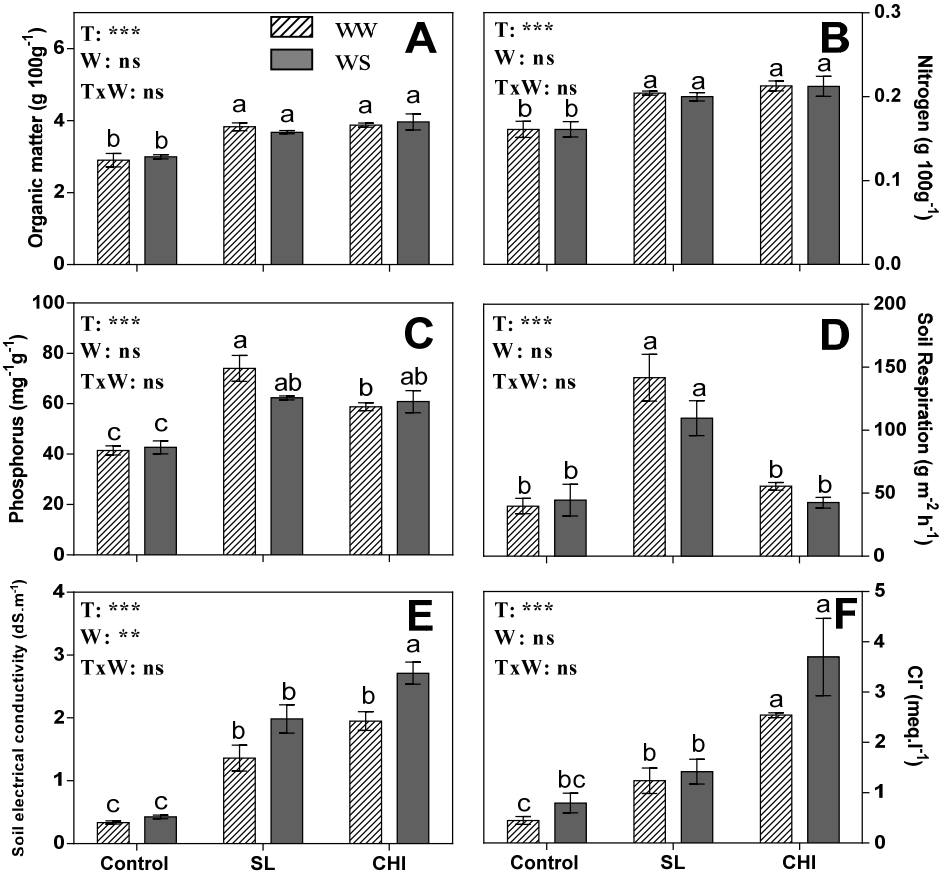

Figure 2. Organic matter concentration (**A**), nitrogen concentration (**B**), phosphorus concentration (**C**), Soil respiration (**D**), soil electrical conductivity (**E**) and chlorine anion (**F**) of maize plants soil with no treatment (Control), waste-based amendment (SL) and chicken manure amendment (CHI). Well-watered conditions are showed in yellow bars (WW) and water stress conditions (WS) in red bars. Bars are means (n = 4) and capped lines are standard errors. Within each graph, bars with the different letter indicate that values are significantly different ($p < 0.05$) Post-hoc Tukey.

Table 2. Relative abundance (%) of bacteria phyla in soil of maize plants of control group, SL treatment and CHI treatment under two watering conditions (WW and WS) Values are means (n = 4) ± S.E. Within treatments, means for each parameter followed by different letter are significantly different Pos Hoc Tukey ($p \leq 0.05$). ANOVA columns represent *p*-value for amendment effect (T), watering conditions (W) and their interaction (TxW). ns not significant; Post-Hoc Tukey * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

	Control		SL		CHI				
Tratamiento	**WW**	**WS**	**WW**	**WS**	**WW**	**WS**	**T**	**W**	**TxW**
Planctomycetota	23.63 ± 0.21a	23.63 ± 1.35a	20.18 ± 0.66ab	18.41 ± 0.89b	18.23 ± 0.91b	19.14 ± 0.51b	***	ns	ns
Bacteroidota	11.8 ± 1.11c	13.03 ± 1.72cb	17.96 ± 0.68ab	20.41 ± 0.62a	19.27 ± 1.58a	16.76 ± 0.68abc	***	ns	ns
Proteobacteria	13.07 ± 0.9b	13.1 ± 0.53b	16.18 ± 1.23b	16.65 ± 2.28ab	22.09 ± 1.15a	18.74 ± 0.99ab	***	ns	ns
Acidobacteriota	15.42 ± 0.68a	15.43 ± 0.82a	11.11 ± 0.83bc	12.54 ± 1.02ab	7.77 ± 0.54c	9.43 ± 0.91bc	***	ns	ns
Verrucomicrobiota	7.57 ± 0.49a	8.74 ± 0.91a	7.69 ± 0.23a	7.94 ± 0.76a	10.28 ± 0.88a	9.11 ± 0.38a	*	ns	ns
Actinobacteriota	7.62 ± 0.6a	8.35 ± 1.38a	6.19 ± 0.27a	5.01 ± 0.74ab	3.15 ± 0.25b	3.34 ± 0.59b	**	ns	ns
Crenarchaeota	3.99 ± 0.3a	3.21 ± 0.22ab	1.79 ± 0.47c	2.27 ± 0.27bc	1.51 ± 0.27c	1.64 ± 0.13c	***	ns	ns
Gemmatimonadota	2.89 ± 0.15a	2.80 ± 0.20ab	2.07 ± 0.15abc	1.85 ± 0.33bc	0.91 ± 0.24d	1.53 ± 0.19cd	***	ns	ns
Firmicutes	0.14 ± 0.07b	0.09 ± 0.02b	0.57 ± 0.02a	0.41 ± 0.18ab	0.40 ± 0.13ab	0.37 ± 0.17ab	*	ns	ns
Bdellovibrionota	0.15 ± 0.03ab	0.14 ± 0.05ab	0.24 ± 0.10ab	0.08 ± 0.05b	0.35 ± 0.11a	0.33 ± 0.08a	*	ns	ns
Methylomirabilota	0.32 ± 0.04a	0.33 ± 0.09a	0.20 ± 0.03ab	0.22 ± 0.08ab	0.04 ± 0.04b	0.09 ± 0.05ab	**	ns	ns

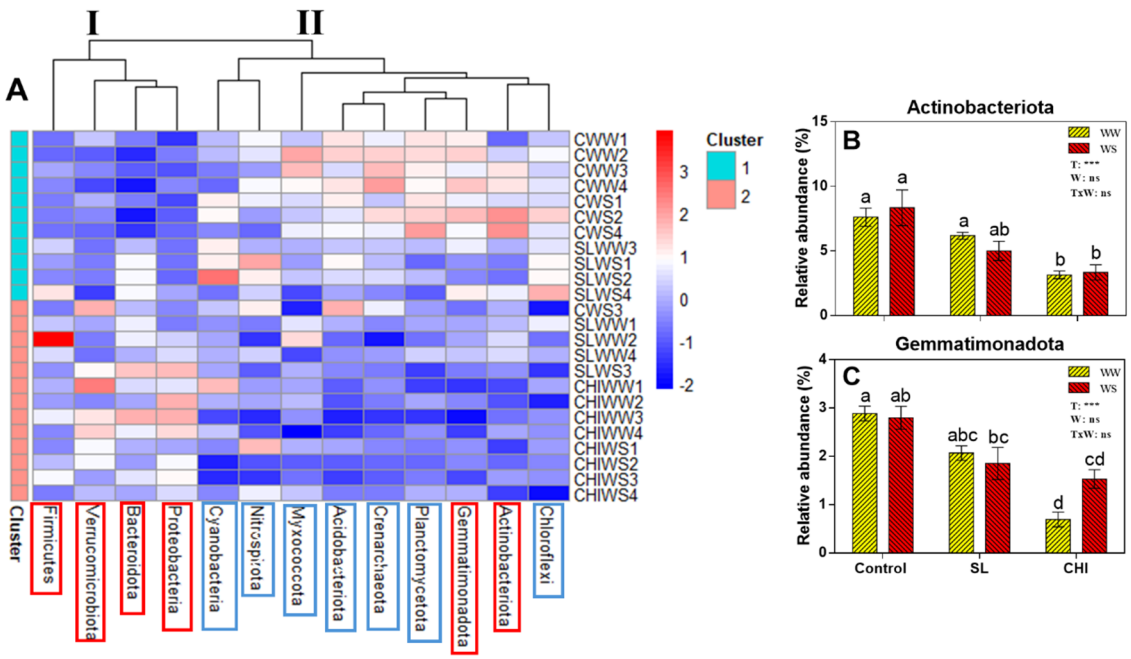

Figure 3. Hierarchical clustering heat map (**A**), relative abundance (%) of Actinobacteriota phylum (**B**) and relative abundance (%) of Gemmatimonadota phylum (**C**), of maize plants soil with no treatment (C), waste-based amendment (SL) and chicken manure amendment (CHI). Well-watered conditions (WW) and water stress conditions (WS). Heatmap shows relative abundance of analysed bacterial phyla were colours ranging from red (increase) to blue (decrease) as shown in the legend. Hierarchical clustering is also shown for whole studied parameters and samples with treatment and conditions using Euclidean distance. Identified phylum as Copiotroph was marked in red, while identified phylum as Oligotroph was marked in blue according to (Stone et al., 2023). Histograms show well-watered conditions in yellow bars (WW) and water stress conditions (WS) in red bars. Bars are means (n = 4) and capped lines are standard errors. Within each graph, bars with the different letter indicate that values are significantly different ($p < 0.05$) Post-hoc Tukey.

Of the 10 fungus phyla analyzed, 8 different phyla were significantly affected by organic amendment application, while only 1 showed significative effects in water conditions and their interaction (Table 3). Under conditions of water stress, both SL and CHI treatments led to an increase in the relative abundance of Aphelidiomycota and Morterellomycota in comparison to the control group. Conversely, under WW, the SL intervention specifically increased the levels of Olpidiomycota and Entomophthoromycota when contrasted with both the control and CHI. In contrast, Glomeromycota a taxonomic group critical for the formation of the rhizosphere and the advancement of crops exhibited a substantial decline under the CHI treatment in relation to the control and SL treatments in well-watered conditions, with CHI demonstrating a consistent reduction of Glomeromycota across both watering scenarios. Furthermore, Ascomycota, the most abundant phylum in the studied soil, showed a tendency to increase under the CHI treatment, particularly in WW.

Table 3. Relative abundance (%) of fungi phyla in soil of maize plants of control group, SL treatment and CHI treatment under two watering conditions (WW and WS) Values are means (n = 4) ± S.E. Within treatments, means for each parameter followed by different letter are significantly different Pos Hoc Tukey ($p \leq 0.05$). ANOVA columns represent *p*-value for amendment effect (T), watering conditions (W) and their interaction (TxW). ns not significant; Post-Hoc Tukey * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

	Control		SL		CHI				
Tratamiento	**WW**	**WS**	**WW**	**WS**	**WW**	**WS**	**T**	**W**	**TxW**
Ascomycota	35.51 ± 2.31 ab	33.03 ± 10.8 ab	26.85 ± 7.64b	35.39 ± 13.18 ab	58.96 ± 8.07a	46.41 ± 1.59 a	*	ns	ns
Glomeromycota	24.81 ± 3.08 a	20.57 ± 3.92 ab	11.86 ± 3.21 bc	6.49 ± 2.55 c	1.55 ± 1.12 d	8.08 ± 3.10 c	***	ns	ns
Basidiomycota	9.88 ± 1.06 a	10.65 ± 1.18 a	6.57 ± 3.38 a	5.48 ± 1.96 a	13.05 ± 6.5 a	11.39 ± 3.59 a	ns	ns	ns
Chytridiomycota	12.93 ± 2.84 a	10.72 ± 3.3 a	10.14 ± 4.55 a	11.6 ± 4.9 a	6.59 ± 1.91 ab	3.09 ± 0.40 b	**	ns	ns
Olpidiomycota	3.56 ± 3.54 ab	0.09 ± 0.09b	19.47 ± 2.8 a	18.07 ± 8.99 ab	0.10 ± 0.01 b	0.10 ± 0.01 b	***	ns	ns
Mortierellomycota	2.04 ± 1.15 b	1.47 ± 0.4 b	1.10 ± 0.55 b	5.57 ± 1.14 ab	9.06 ± 1.61 a	10.45 ± 2.57 a	***	ns	ns
Mucoromycota	6.89 ± 3.38 a	10.92 ± 10.55 a	3.98 ± 1.28 a	0.75 ± 0.22 a	0.11 ± 0.06 a	0.19 ± 0.03 a	ns	ns	ns
Rozellomycota	3.85 ± 0.5 a	3.62 ± 1.42 ab	2.00 ± 0.57 ab	1.91 ± 0.92 ab	0.86 ± 0.31 b	1.07 ± 0.24 b	**	ns	ns
Aphelidiomycota	0.01 ± 0.01 b	0.01 ± 0.01 b	1.14 ± 0.5 b	1.36 ± 0.43 b	1.78 ± 0.29 b	19.22 ± 3.51 a	**	*	*
Entomophthoromycota	0.05 ± 0.03 a	0.07 ± 0.05 a	0.11 ± 0.05 a	0.25 ± 0.11 a	0.02 ± 0.01 a	0.04 ± 0.01 a	*	ns	ns

Fungal community composition was also assessed using heatmap visualization and hierarchical clustering (Figure 4A). However, the six treatment combinations (two watering regimes and three amendment treatments) did not segregate into distinct clusters, indicating a limited effect of either amendment type or water regime on the overall fungal community structure. Additionally, no clear tendencies were observed in the different major cluster's groups of fungi phyla. Although no general aggrupation among all phyla was observerd, Aphelidiomycota and Morterellomycota groups tended to increase under CHI treatment, mainly under water-stress conditions while Olpidiomycota and Entomophthoromycota tended to increase under SL treatment under well-watered conditions. On the other hand, a relevant group in soil related to the rhizosphere formation and crop development defined as Glomeromycota almost disappeared under CHI treatment compared to control and SL amendment in well-watered conditions. Additionally, CHI decreased the relative abundance of this group under both watering conditions compared to control group (Figure 4B).

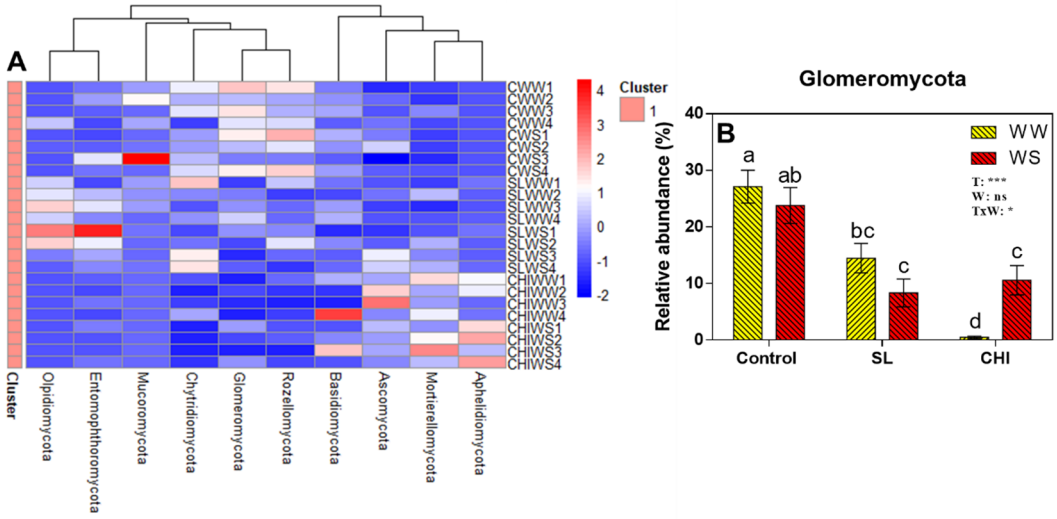

Figure 4. Hierarchical clustering heat map (**A**), relative abundance (%) of Glomeromycota phylum (**B**) of maize plants soil with no treatment (Control), waste-based amendment (SL) and chicken manure amendment (CHI). Well-watered conditions (WW) and water stress conditions (WS). Heatmap shows relative abundance of analyzed bacterial phyla were colours ranging from red (increase) to blue (decrease) as shown in the legend. Hierarchical clustering is also shown for whole studied parameters and samples with treatment and conditions using Euclidean distance. Histograms show well-watered conditions in yellow bars (WW) and water stress conditions (WS) in red bars. Bars are means (n = 3) and capped lines are standard errors. Within each graph, bars with different letters indicate that values are significantly different ($p < 0.05$) Post-hoc Tukey.

3.4. Clustering and multifactorial analysis of the effect of organic amendment and watering conditions on plant physiology and soil microbial phyla

Of the 95 analysed parameters, 75 were significantly affected by amendment treatment, irrigation conditions or their interaction. To reduce complexity of the multivariate data and identify patterns, principal component analysis (PCA) was performed (Figure 5A). The PCA revealed that the first principal component (PC1) explained 46.3% of the total variation between amendments and water regimen. Remarkably, a clear differentiation between amendments could be done. Besides. the loading plot revealed that the discrimination of samples by the second principal component (PC2) (28.1% of the total variance) was, in part due to soil parameters such as soil N and Cl- concentration, organic matter and conductivity or minerals such as Zn, Na or Cu. (Figure 5A).

To better understand the effects of organic amendment applications and watering conditions in the different obtained bacterial phyla, a PCA-Biplot of analysed bacterial phyla from relative abundance values was performed (Figure 5B). The first principal component (PC1) explained 13.4% of the total variance while the second principal component (PC2) explained 47.7% of the total variance. Samples were grouped in circle colours according to organic amendment and irrigation conditions: Control (blue), SL amendment (orange) and CHI amendment (green) under WW conditions, while under WS conditions: Control (darker blue), and SL amendment (red) and CHI amendment (violet) These results show the evidence that some groups differences are due mainly to organic amendment application. Also, this allowed us to detect some relevant bacterial groups related to soil quality and the behaviour of beneficial groups. Vectors pointing to different clustered groups indicates positive correlation. According to these different phyla was found more positively correlated to control and SL treatments while other phyla were more positively correlated to CHI treatment. Clear difference between Control treatment and CHI treatment could be observed as separated groups.

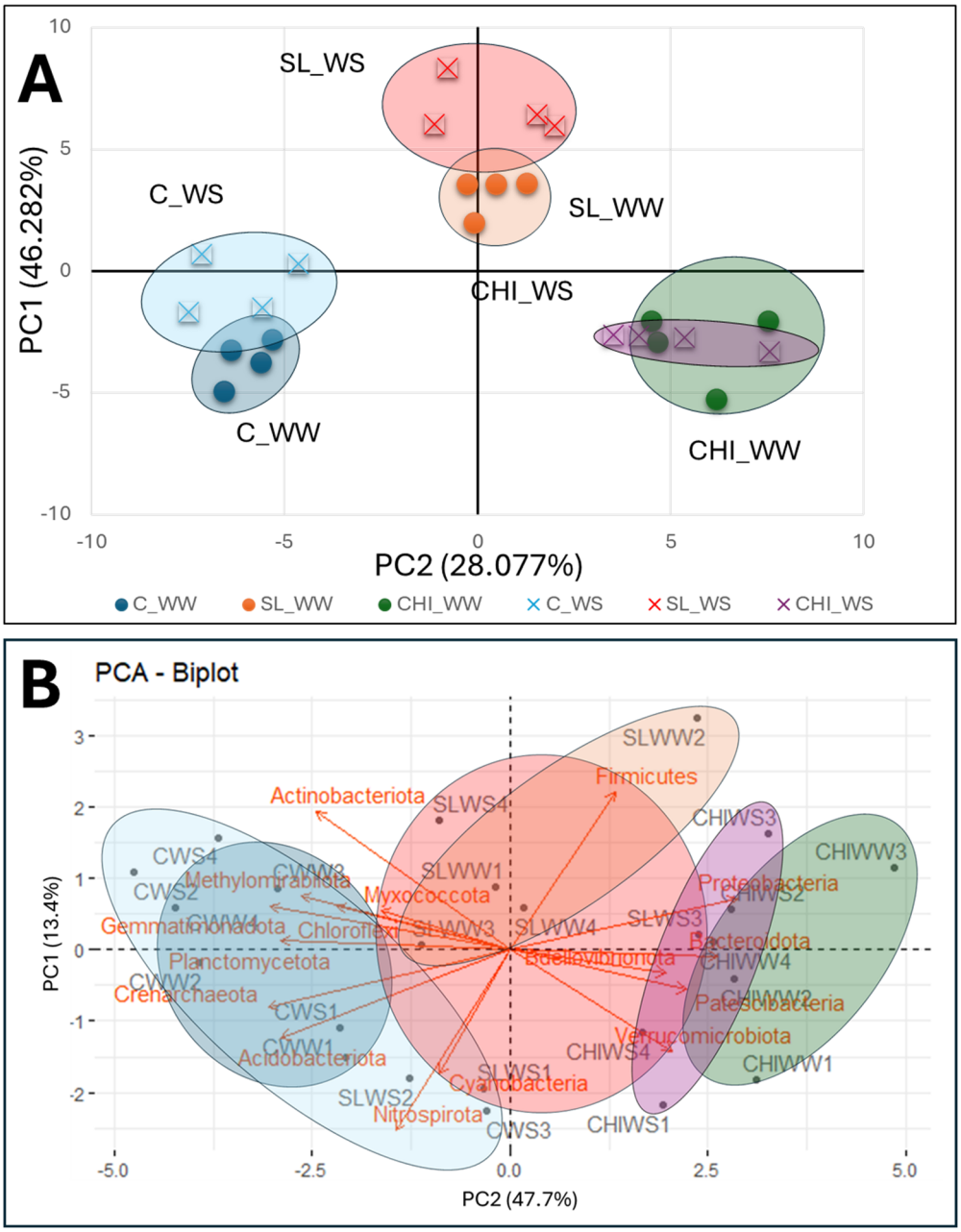

Figure 5. Principal component analysis (PCA) (**A**) and bacterial phyla PCA-Biplot (**B**), of maize plants soil with no treatment (C), waste-based amendment (SL) and chicken manure amendment (CHI). Well-watered conditions (WW) and water stress conditions (WS). PCA shows individual scores grouped in control treatment with well-watered conditions (C_WW) in dark blue circles, control treatment with water stress conditions (C_WS) in light blue crosses, SL treatment with well-watered conditions (SL_WW) in orange circles, SL treatment with water stress conditions (SL_WS) in light red crosses, CHI treatment with well-water stress conditions (CHI_WW) in green circles and CHI treatment with water stress conditions (CHI_WS) in purple crosses. The biplot shows individual scores for analysed bacteria phyla. Colored concentration ellipses (size determined by a 0.95-probability level) show the observations grouped same as PCA. Vectors pointing in similar directions indicate positively correlated variables, vectors pointing in opposite directions indicate negatively correlated variables.

4. Discussion

4.1. Contrasting effects of organic amendment application on plant growth and physiology

Organic amendments serve as a promising approach to enrich soils with vital nutrients, enhance moisture retention, foster beneficial microbial activity and improve soil structure and aeration (Matisic et al., 2024). However, our results demonstrate that the effects of organic amendments extend beyond nutrient supply and are largely determined by their capacity to modify soil–plant–microbiome responses. The sludge-based amendment (SL) increased soil organic matter, nutrient availability, and microbial activity, creating conditions that favored nutrient cycling and resource acquisition by maize plants. These changes were associated with higher photosynthetic performance, as indicated by increases in PSII efficiency, electron transport rate, and net CO_2 assimilation, resulting in greater biomass accumulation

specifically, under WW conditions. Overall, significant treatment × watering interactions were detected for all analyzed variables, indicating that the physiological and growth responses of maize to water stress depended on the type of organic amendment applied (Figure 1). Although SL-treated plants showed higher photosynthetic performance than the control, absolute photosynthetic rates measured across treatments were lower than those typically reported for maize grown under optimal conditions. This suggests that plant carbon assimilation may have been constrained by factors independent of water availability probably related to reduced availability of micronutrients such as Zn (constraining photosynthetic activity, chlorophyll formation and electron transport processes (Mattiello et al., 2015). Interestingly, the improved performance of SL-treated plants under well-watered conditions was not associated with major increases in foliar nutrient concentrations except for sulfur (Table S3). Instead, these plants exhibited greater accumulation of soluble sugars and amino acids involved in carbon and nitrogen metabolism, including fructose, glutamine, asparagine and alanine, together with higher photosynthetic activity. This suggests that SL enhanced nutrient turnover and assimilation processes rather than nutrient accumulation. However, this metabolically active state also appeared more sensitive to water limitation. Under WS conditions, SL-treated plants exhibited higher drought-stress indications, such as a reduction of photosynthesis and stomatal conductance alongside a notable accumulation of amino acids and abscisic acid (ABA) according to previous studies (Aslam et al., 2022; Hu et al., 2023), alongside decreases in starch content compared to the control (Table 1). Therefore, while SL amendments promote plant growth in favorable conditions, their advantages might be offset under water-stress situations. In line with previous research; greater biomass produced in optimal environments results in elevated transpiration rates, requiring a larger distribution of metabolic resources towards stress response mechanism. (Aranjuelo et al., 2007). In contrast, chicken manure (CHI) amendment consistently showed negative effects on maize growth. Despite CHI boosting soil organic matter and certain nutrients like nitrogen and phosphorus, it also increased soil salinity due to elevated ion chloride levels and electrical conductivity, which likely led to phytotoxicity and decreased photosynthetic capacity (Figure 2). The absence of increments in soil respiration is also indicative of potential constraints of CHI on microbial metabolic processes. Stress is also observable in physiological parameters of the maize plants, which shows declines in vital protective pigments, including anthocyanins and flavonoids. In contrast, despite the greater accumulation of several nutrients in CHI-treated plants, particularly P, Mg, Mn and S, these plants also exhibited substantially higher Na concentrations (Table S3). Together with the elevated soil electrical conductivity and chloride content, this suggests that nutrient enrichment alone was insufficient to sustain growth and that salt-related effects likely limited biomass accumulation and physiological performance. interestingly, under water-stress conditions, CHI-treated plants exhibited higher net CO_2 assimilation and electron transport rates despite the absence of significant changes in stomatal conductance, Fv/Fm and ΦPSII. Elevated salinity associated with CHI application, evidenced by increased soil electrical conductivity, chloride concentrations and foliar Na accumulation, induced an early stress acclimation response. Such pre-conditioning may have enhanced the capacity of plants to maintain photochemical performance under subsequent drought exposure, reducing the physiological impact of water limitation. Thus, in the same manner as others has found (Aranjuelo et al., 2006), plants adapted early to stress showed reduced impact of a second, in this case, WS conditions. These findings are consistent with prior reports of growth inhibition due to salinity and highlight the necessity of considering amendment-specific properties when determining fertilization approaches.

4.2. Impact of different organic amendments application in soil microbial communities

By grouping bacterias based on their reactivity to carbon sources (Stone et al. 2023) we detected some relevant insights (Figure 3). In soils treated with SL, copiotrophic bacteria such as Firmicutes, Bacteroidetes, and Proteobacteria saw an increase, whereas oligotrophic bacteria experienced a decline, including Acidobacteria, Nitrospira, Crenarchaeota, and Planctomycetota, (Table 2). Beyond the compositional shifts themselves, these microbial responses provide a potential mechanistic explanation for the contrasting maize performance observed among treatments. The enrichment of copiotrophic taxa in SL-amended soils suggests increased decomposition of organic substrates and nutrient turnover, which is consistent with the higher microbial respiration, improved nutrient availability, and enhanced biomass accumulation observed in maize. Under WW conditions, these changes likely supported higher photosynthetic activity and plant growth. However, under drought conditions, the larger biomass supported by SL may have increased plant water demand, contributing to stronger reductions in stomatal conductance and photosynthesis when water became limiting in line with Cui et al. (2023) found that organic amendments increase the dominance of copiotrophic bacteria phyla. In contrast, CHI amendment generated a distinct soil environment characterized by increased electrical conductivity and chloride accumulation. These conditions were accompanied by reductions in important copiotrophic groups such as Actinobacteriota and Gemmatimonadota, microorganisms associated with organic matter turnover

and nutrient cycling (Bao et al., 2021). The simultaneous occurrence of salinity stress and reduced abundance of beneficial microbial taxa may have constrained nutrient acquisition and photosynthetic performance, thereby contributing to the lower biomass production observed in CHI-treated plants. Consequently, the negative effects of CHI on maize growth appear to result from the combined action of soil chemical stress and microbial community disruption (Egamberdieva et al., 2010; Yang et al., 2021). Furthermore, the relatively stable presence of Gemmatimonadota in WS conditions indicates a stronger adaptation of this group to arid environments, as previously seen in (Mucsi et al., 2024).

Among the analyzed fungal phyla, Ascomycota (the predominant group) along with other naturally occurring soil fungi such as Morteriellomycota and Aphelidiomycota, increased with the application of organic amendments, particularly in CHI-treated soils (Table 3). This was found in Luo et al. (2023) and Tarin et al. (2021). In contrast, the reduction of Glomeromycota (Figure 4B) is particularly relevant in the context of drought tolerance because arbuscular mycorrhizal fungi contribute to plant water and nutrient acquisition under water-limited conditions. Previous research has shown that fertilization or enhanced soil nutrient availability can reduce plant reliance on AMF, resulting in a decrease in their populations within the rhizosphere (Bhadalung et al., 2005; Lu et al., 2022). Therefore, the decline of this fungal group in amended soils, particularly under CHI treatment, may have weakened plant-microbe interactions that normally support maize adaptation to drought. This finding suggests that amendment-induced changes in microbial communities do not necessarily translate into increased drought resilience and that the maintenance of beneficial symbioses may be an important factor determining amendment effectiveness under water stress (Zhang et al., 2024).

5. Conclusions

Plant responses to organic amendments are shaped by a network of interactions linking soil chemistry, microbial activity, and plant metabolism, making amendment identity a potentially important determinant of crop performance under drought. In this study, sludge waste amendment improved soil fertility, stimulated copiotrophic bacterial groups associated with organic matter decomposition and nutrient cycling, thereby enhancing photosynthetic performance, metabolism, and biomass production particularly under well-watered conditions. However, the greater productivity promoted by this amendment was accompanied by increased sensitivity to water limitation, as evidenced by stronger reductions in gas exchange and higher accumulation of stress indicators such as ABA. In contrast, the chicken manure amendment increased soil salinity and altered microbial community composition, reducing the abundance of several beneficial microbial groups involved in nutrient cycling and plant–soil interactions. These changes were associated with lower plant growth and photosynthetic performance, suggesting that amendment-induced salinity may have partially counteracted partially offset the potential benefits of increased nutrient availability. Furthermore, the reduction of beneficial microbial taxa, including groups linked to nutrient turnover and plant resilience, may have contributed to the poorer performance of maize under this growth conditions. The present study highlights that improvements in soil fertility do not necessarily confer greater drought tolerance. Rather, the agronomic value of organic amendments appears to depend on their capacity to shape the soil environment as a whole, including microbial community structure and physicochemical conditions. These results emphasize that amendment composition is a critical determinant of crop performance under water limitation and should therefore be carefully considered when designing sustainable nutrient management strategies for drought-prone agricultural systems.

Supplementary Materials

The additional data and information can be downloaded at: https://media.sciltp.com/articles/others/2611251834437593/PlantEcophys-26070158-SI.pdf.

Author Contributions

Conceptualization, I.A.; Data curation, P.J.P., David Soba; Investigation, P.J.P., D.S. and I.J.; Methodology, P.J.P., D.S.; Resources, I.A.; Supervision, I.A.; Writing—original draft, P.J.P., I.J.; Writing—review & editing, I.A. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

The original contributions presented in this study are supported in the article; further inquiries can be directed to the corresponding authors.

Conflicts of Interest

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Use of AI and AI-Assisted Technologies

During the preparation of this work, the author(s) used Microsoft Copilot service to improve the clarity and grammar of the English. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

Peer Review Statement

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