



Impact of Different Organic Amendments on Soil Health and Banana Plant Performance

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Abstract

Purpose Organic amendments (OAs) from agricultural and livestock residues are usually used to improve soil fertility in crop systems, however, the knowledge of the banana agroecosystem remains limited. The aimed of this study was to evaluate and compare the effect of four OAs commonly used in Canary Islands, on soil health and plant performance.

Methods A two-year greenhouse experiment was carried out over a complete crop cycle. The treatments included cow manure, chicken manure, compost, pelletised compost, and a non-amended control. Soil chemical properties, microbial activity, plant growth, and plant nutrient content were monitored across the experiment.

Results All OAs improved soil parameters relative to the control, with differing impacts among treatments. Chicken manure markedly enhanced soil microbial activity (increasing induced respiration and the abundance of bacterial, actinobacterial, and fungal populations), increased phosphorus and electrical conductivity, and reduced pH (by up to 1.5 units). Cow manure increased soil nutrient availability (especially nitrogen and phosphorus) and stimulated basal microbial respiration. Compost promoted fungal abundance and increased total and oxidisable organic matter throughout the crop cycle, whereas pelletised compost showed variable effects. In terms of plant development, chicken and cow manure significantly improved height, biomass, pseudostem circumference, and leaf area. Chicken manure outperformed cow manure in several yield-related parameters, including bunch and hand weight, number of fingers/hand, finger size, and rachis diameter. At harvest, chicken manure also showed the highest uptake of calcium, iron, manganese, nitrogen, potassium, sodium, and zinc.

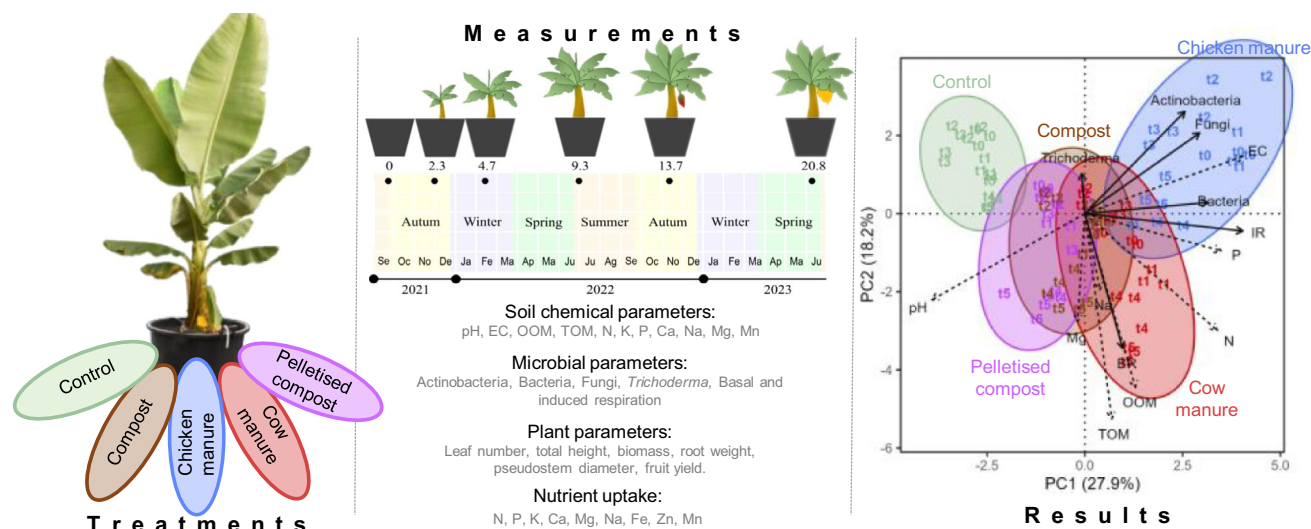
Conclusions These results highlight the different effects of each OAs and their specific potential to improve soil biological parameters, nutrient availability, and banana yield as effective tools to enhance agroecosystem sustainability.

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Graphical Abstract



Keywords Organic amendments · Cow manure · Chicken manure · Compost · Pelleted compost · Soil microorganisms

1 Introduction

Banana (*Musa* spp.) is a key crop in the global fruit trade, with a significant impact on international markets (FAO 2024). Global production is projected to grow at an annual rate of 1.5%, reaching 135 million tonnes by 2028 (Liu et al. 2019). This crop is predominantly cultivated in tropical regions such as India, China, and Brazil (FAO 2024), and in subtropical areas like the Canary Islands (Spain), where it is particularly important (Bebber 2023). In 2022, banana yields in the Canary Islands reached approximately 450,000 tonnes, accounting for about 64% of the total production in the European Union (FAO 2023; ISTAC 2022). Likewise, bananas are an important source of income for local farmers and contribute significantly to employment and the regional economy.

Bananas are typically cultivated under conventional monoculture systems that require high inputs of water and inorganic nutrients. To achieve high yields, farmers often apply excessive amounts of fertilizers, especially nitrogen, surpassing the plants' nutritional requirements (Mahecha-Vásquez et al. 2017). These practices result in nutrient imbalances, soil acidification, and the leaching of essential cations such as calcium and magnesium, which are important for fruit quality (Guo et al. 2010; Zhang et al. 2020). They also favour the accumulation of autotoxic substances, disrupt the native microbiota, increase the proliferation of soilborne pathogens, reduce organic matter content, decrease water retention capacity, and contribute to the degradation of soil structure (Roldán et al. 2005; Zhao et al. 2018). Consequently, this type of intensive agriculture

causes significant negative impacts on the soil ecosystem (Wahome et al. 2023). In view of this situation, identifying and implementing alternative strategies to improve and restore soil health has become increasingly important. In this context, the use of organic amendments (OAs) provides a sustainable alternative to chemical fertilizers by improving soil fertility through a balanced nutrient supply, increasing organic matter content, enhancing microbial diversity, and promoting beneficial microbial activity (Bonanomi et al. 2018; Debosz et al. 2002; McGeehan 2012; Sradnick et al. 2013; Xia et al. 2017). Studies have shown that various OAs, such as manure (cow, chicken, pig, and goat), compost, and biochar, can enhance soil properties in banana crops (Sunarta and Trigunasih 2017; Vanilarasu and Balakrishnamurthy 2014; Zhang et al. 2020). For instance, Zhang et al. (2019) observed enhanced soil organic carbon levels and greater microbial diversity in banana crops amended with filtered sugar factory sludge and plant residues. Similarly, Vanilarasu and Balakrishnamurthy (2014) demonstrated that organic manures enhanced mineralization, nutrient solubilisation, and microbial activity. Heck et al. (2019) also observed increased soil respiration following the application of composted sewage sludge, biochar, shrimp shells, and mussel shells. Beyond improving soil properties, OAs also reduce soilborne pathogens. For instance, Tabarant et al. (2011) demonstrated that sugarcane bagasse and sewage sludge reduced nematode populations. Similarly, several studies have shown that certain OAs, such as chicken and sheep manure, shrimp shells, cattle manure compost, and pig manure compost, can reduce the incidence of *Fusarium* wilt (*Fusarium oxysporum* f. sp. *cubense*), one of the most

devastating diseases affecting banana crops (Heck et al. 2019; Shen et al. 2013; 2015; Sun et al. 2018; Xiong et al. 2017). In contrast, some amendments have shown variable or even negative results in controlling the disease (Nasir et al. 2003; Lang et al. 2012). Therefore, several authors have explored the enrichment of these amendments with specific microorganisms to create bioorganic fertilizers for disease control (Dita et al. 2018).

Banana production in the Canary Islands is generally characterised by effective management of soil organic matter. In most farms, the incorporation of organic residues from harvested plants and the application of OAs (such as manures or composts) is a common practice, especially in agroecological systems. Typically, farmers apply these amendments based on a general dosage (approximately 3–4 kg per plant or 2–3 kg per m²), and select the type of amendment (different types of manure or compost) according to their personal experience, market availability, or economic possibilities. A recent survey (Perera González et al. 2023), reported that the 58% of banana producers apply manure, while 15% use compost. Regarding frequency, manure is mostly applied every three years (33% of surveyed farmers), and compost is applied at the same interval by 53% of those using it (Perera González et al. 2023). However, despite being a common practice in the Canary Islands, there are limited specific studies comparing the effects of different OAs on the chemical and microbiological properties of the soil, as well as on plant development (Alvarez et al. 2001). To address this issue, we hypothesized that the application of OAs commonly used in the Canary Islands would improve soil fertility and microbial activity, leading to enhanced nutrient uptake and plant growth compared with unamended soil, with different effects on the chemical and microbiological properties of the soil depending on the type of amendment used. Based on this hypothesis, the study aimed to evaluate the effects of four different OAs on soil quality and banana plant performance. Considering the crucial role of microbial populations in soil health and crop sustainability, we assessed the impact of each amendment on the dynamics of soil microbiota (bacteria, actinobacteria, and filamentous fungi) and on biomass metabolic activity over the course of the plant cycle. In addition, soil chemical parameters, nutrient uptake, and plant development were evaluated.

2 Material and Methods

2.1 Plant Material

Banana plants (*Musa acuminata* AAA Colla cv. ‘Gruesa Palmera’) were initially provided as seedlings from in vitro cultures. Once at the Canary Institute of Agricultural

Research (ICIA) facilities (Santa Cruz de Tenerife, Spain), the plants were transferred to a greenhouse with a natural daylight photoperiod and maintained at a controlled temperature of 28 °C and 70% humidity. Over the course of three months, the plants were carefully monitored every two to three days and irrigated manually according to pot size, plant growth, and evaporation rate, aiming to maintain the soil close to field capacity and to ensure uniform conditions among treatments during the acclimation phase. Once the plants reached a sufficient stage of development, they were transplanted into pots (Ø = 20 cm, 2 L) filled with a sterile substrate mixture composed of equal parts of soil, peat, and volcanic basaltic pyroclastic material between 4–8 mm of size (or “picón”, as it is locally called) (1:1:1, v:v:v). This step marked the end of the acclimation period. The plants remained in these pots for an additional two months under the experimental conditions described below, until being transplanted to the experimental setup.

2.2 Soil and Organic Amendments

2.2.1 Soil

The soil used in this study was classified as Andisol, representing the typical soil of banana farms in Tenerife Island (Tejedor et al. 2009). More than 4.5 tonnes of soil were obtained from the Valle de La Orotava (northern Tenerife) through a private company (Estévez Méndez S.L., Tenerife, Spain) specialised in soil extraction for agricultural use in “sorribas”. This is a common practice in banana-growing regions of the Canary Islands, where fertile soil from mid- and high-altitude areas is transported to coastal terraces established on volcanic substrates. In this study, the soil was extracted from the upper horizons of agricultural fields in the mid-altitude areas during the spring of 2021, under moderate temperature and humidity conditions. The soil chemical parameters are shown in Table S1.

2.2.2 Organic Amendments

Four OAs widely used in the Canary Islands banana sector were applied: 1) chicken manure, 2) cow manure, 3) compost, and 4) pelletised compost (referred to as pellet). The chicken and cow manures were obtained from local livestock farms that regularly supply manure to banana plantations, the compost was provided by the company Suna 2000 S.L. (Tenerife island, Spain), a supplier of agricultural compost, and the pelletised compost by Compost Majoreros S.L. (Fuerteventura island, Spain), a company specialising in pelletised compost for agricultural use. All OAs were selected for their common application and relevance

in banana crops in the Canary Islands. The OAs chemical parameters are shown in Table S2.

2.2.3 Addition of Organic Amendments

A total of 45 pots with a capacity of 170 L ($\varnothing=73$ cm) each were used. To prepare the soil–OA mixtures, over 4.5 tonnes of soil were thoroughly mixed after removing any plant residues. To achieve proper aeration of the mixture in the pots, volcanic basaltic pyroclastic material (particle size: 4–8 mm) was mixed with the soil in a 0.4:0.6 ratio (basaltic pyroclastic:soil, v:v). To ensure the correct homogeneity of the mixture, soil, volcanic ash and amendments were mixed using a mechanical homogenising machine (concrete mixer). In order to standardize and unify the application of each amendment, the total organic matter (TOM) content was used as the dosage criterion (Moral Herrero and Muro Erreguerena 2008). Based on this approach, the usual application rates in the Canary Islands for the addition of compost in banana crops were taken as a reference, equivalent to an initial addition of 1.5% TOM to the soil. Taking this parameter into account, the percentage of TOM, moisture content, and density of each amendment were considered to determine the mixture required in each case (Table S2). Consequently, the amount of organic amendment added to each pot (170 L) was as follows (kg DM): Chicken manure: 4.4, Cow manure: 4.5, Compost: 5.6, Pelletised compost: 6.4.

2.3 Experimental Design and Sample Collection

The pot experiment was carried out in a greenhouse (11.56 m \times 14.21 m: 164 m²) located at 30° 35.88" N, 16° 23' 18.53" W and 351 m above sea level, in a typical subtropical climate, within the facilities of the ICIA, Tenerife, Spain. The experiment was conducted over a two-year period, from August of 2021 to June 2023. The temperature and humidity variations in the greenhouse during this period are shown in Fig. S1. A total of five treatments (four organic amendments and one unamended control) were established, each with nine replicates, giving a total of 45 pots arranged in a randomized complete block design.

After one month of stabilisation of the soil/amendment mixture in the pots, a banana seedling previously acclimatized (see 2.1. *Plant material*), was planted in the centre of each pot. All seedlings used were of similar height and leaf age. Irrigation (Table S3) was carried out by drip system, and the volume of water used was adequate to maintain the soil at its field capacity and to provide the plants with optimal hydric conditions.

2.4 Soil and Plant Collection

Soil samples were taken with an auger at a depth of 25 cm at six intervals during the experiment: 0 months (pre-planting), 2.37 months, 4.77 months, 9.33 months, 13.70 months, and at the end of the experiment at 20.80 months. (Fig. S1). Soil sampling was conducted by taking four subsamples per pot (one from each side of the plant) in each treatment. Subsequently, the subsamples (12) from three pots were combined to form a composite sample, resulting in a total of three composite samples per treatment at each sampling interval. This approach ensured that all nine replicates were adequately represented in each of the composite samples for each treatment. The composite samples (6 kg) were then homogenized (through a 2-mm sieve) and processed for subsequent analysis. Half of soil sample (3 kg) was used for determining soil chemical parameters, culturable microbial populations, and soil respiration (see *Chemical Analysis of Soil and Amendments*, and *Soil Microbial analysis* section), while the other half of soil sample was stored at -80 °C for future analysis.

At each sampling time, the following parameters were evaluated: culturable microbial populations, basal and induced biomass respiration, soil chemical parameters, and plant growth. At the end of the experiment, biological and nutritional parameters of banana plants were analysed (See *Plant Analysis* section).

2.5 Chemical Analysis of Soil and Amendments

Five hundred grams of each soil sample from each treatment and sampling time were processed to measure the following chemical parameters: 1) pH in water (1:5, w:w) following the procedure based on UNE-ISO 10390:2012 (UNE-ISO 10390:2012 2012), 2) electrical conductivity (EC) in extract (1:5, w:w, in water; mS cm⁻¹, μ S cm⁻¹), 3) oxidisable organic matter (%) (OOM) by oxidation with potassium dichromate and titrated with Mohr's salt (Walkley and Black 1934), 4) total organic matter (%) (TOM) by calcination (Brame and Lefevre 1977), 5) total nitrogen (%) (N) via the Kjeldahl method (Bremner 1996), 6) phosphorus (mg kg⁻¹) by colorimetric method (Olsen et al. 1954), 7) calcium, magnesium, sodium, and potassium (mEq kg⁻¹) were extracted using ammonium acetate and determined by flame atomic absorption spectrophotometry (Bower et al. 1952).

For the OAs, the following parameters were analysed: 1) TOM, 2) total organic carbon (TOC), 3) nitrogen, 4) C/N ratio, 5) pH in water (1:5), and 6) electrical conductivity. All laboratory procedures were carried out by the ICIA Laboratory Unit, located within the same facilities where the greenhouse experiment was conducted.

2.6 Soil Microbial Soil Analysis

A standard serial dilution and spread-plate method was used to quantify the culture-dependent microbial populations. Briefly, 20 g of each soil samples were mixed in 180 mL solution (0.85% KOH) and homogenized for 20 min at 120 rpm using a JP Selecta Rotabit (Spain) shaker. Serial dilutions (1:10) were made and 100 μ L from each dilution was aseptically spread on Petri plates with: Dichloran Rose Bengal Chloramphenicol Agar (DRBC, Condalab, Madrid, Spain) to quantify filamentous fungi and *Trichoderma*, Plate Count Agar (PCA, Scharlau, Madrid, Spain) for total aerobic bacteria and Gauze No.1 medium (Gauze 1958) for actinobacteria. To avoid the growth of abundant and fast-growing soil bacteria that would hinder the quantification of actinobacteria (slower growing) by overgrowth, a thermal pre-treatment heat at 100 °C (dry heat) for 1 h was applied to the soil sampled before the serial dilutions (Williams and Cross 1971). Once the plates were plated, they were incubated at 25 °C for 24–48 h (bacteria) and 5–7 days (filamentous fungi, actinobacteria and *Trichoderma*). The colonies of each type of microorganism were counted and expressed as colony forming units per gram of soil (cfu g⁻¹). Each analysis was performed in triplicate.

Soil basal and induced respiration were measured following the methodology of Anderson (1982). Briefly, soil samples (50 g) were wetted to 50% of their water retention capacity and stabilised for 15 days in 200 ml flask placed inside 3 L containers. Subsequently, a flask containing 20 ml of sodium hydroxide (0.5 M) was placed inside the container and hermetically sealed. The respiration (CO₂ production) was assessed after incubation the sample for 7 days at 25 °C, followed by titration of sodium hydroxide with hydrochloric acid (0.5 M). The induced respiration was performed with the same method as above (basal respiration) but by adding a sterile 8% glucose solution to the soil (2.5 ml per sample). After the samples were incubated at 25 °C for 2.5 days, the sodium hydroxide (0.5 M) was titrated with hydrochloric acid (0.5 M). The experiments were carried out in triplicate and a soil-free treatment was used as a control. The results were expressed in mg C-CO₂ kg⁻¹ dry soil per day.

2.7 Plant Analysis

At the end of the experiment (20.8 months) the plants were harvested and separated into roots, corm (rhizome or head), pseudostem and leaves. Fresh and dry weights (kg) of the different plant parts were recorded. Samples were weighed, chopped, oven-dried at 70 °C for 72 h to determine the water content. The total length, and the length of the different parts (pseudostem, radicular system, etc.) were measured.

The circumference of the pseudostem was determined at a height of 10 cm above the soil surface. The number of green leaves (> 50% green area), the length and width of the third leaf and total leaf area (expressed in m²) were recorded. The yield was analysed by different bunch parameters: weight, length, number of bunch hands and number of fingers.

The nutritional status of the plants was assessed by sampling leaves from each treatment. Samples (5 g) were dried (60 °C, 24 h), ground (< 1 mm) (Foss Cyclotec 1093), and mineralised (Carbolite ELF 11/6) at 500 °C with HCl (2 M). Calcium, magnesium, sodium, potassium, zinc, iron, copper and manganese, were determined by atomic absorption spectroscopy (Thermo Scientific Series S4). Nitrogen was determined by the Kjeldahl method (Selecta M-24) and phosphorus by UV–visible Absorption Spectrophotometry. All the parameters were determined following the procedure of Ministry of Agriculture, Fisheries and Food (MAPA) of Spain (1986) by the ICIA Laboratory Unit.

2.8 Statistical Analysis

The statistical analyses were conducted using the ‘R’ software version 4.1.0 (R Development Core Team 2021) and the integrated development environment RStudio (R Studio version 1.4.1103, RStudio Team 2016, Boston, MAA). Figures were obtained using the package ggplot2 (Wickham 2016). Analysis of variance (ANOVA) (considering significant differences when the *p*-value < 0.05), T-tests and post hoc tests were applied when appropriate, as indicated in the corresponding Figure legends. The ordination of samples based on microbial counts and soil parameters over time was visualized using Principal Components Analysis (PCA). This allowed for the identification of patterns and clustering within the dataset. Normality and homoscedasticity of the residuals were tested with Shapiro–Wilk and Levene’s tests, respectively. If required, data (microbial counts) were transformed to meet the model assumptions.

3 Results

3.1 Impact of Organic Amendments on Soil Chemical Parameters

The effect of the addition of four OAs on soil chemical properties over a period of two years in a greenhouse experiment with banana plants was studied. The chemical characterization of the OAs used in this study, including nutrient content, is provided in Supplementary Table S2. This information is crucial for understanding the potential contributions of the amendments to soil fertility and plant growth, particularly in terms of nutrient availability. Six samples were collected

over time, and all OAs showed significant effects compared to the control treatment (no amendment) across all assessed parameters, demonstrating significant improvements in soil quality (Table S4).

The addition of OAs primarily affected the percentages of TOM and OOM, as well as the amount of nitrogen, phosphorus and magnesium (Fig. 1). Most OAs significantly increased TOM compared to the control at 0, 4.77, 9.33, 13.70, and 20.80 months, except for

chicken manure, which showed significant differences with the control only at the beginning of the experiment (0 and 2.37 months). In contrast, cow manure showed significant differences at all sampling times, including at 2.37 months (Fig. 1a). The compost treatment had the highest TOM content at 0, 4.77, 9.33, and 13.70 months, while cow manure and pelletised compost recorded the highest TOM levels at the end of the experiment (20.80 months).

Similar results were observed for OOM (Fig. 1b). Compost treatment consistently showed the highest OOM percentage across sampling times, with significant differences from other treatments at 0 and 9.33 months, while at the rest of the sampling times the percentages were not significantly different from the cow manure treatment. In contrast, chicken manure showed the lowest OOM percentage of all the amendments treatments at all sampling times (except for the first sampling). The control remained significantly lower than all treatments throughout the experiment, except at time 0, when values were similar to pelletised compost (Fig. 1b). Overall, cow manure and compost increased TOM and OOM the greatest, while chicken manure had the least effect.

Soil nitrogen content was significantly higher in all amended treatments compared to the control across all sampling times (Fig. 1c). Cow manure showed the highest levels

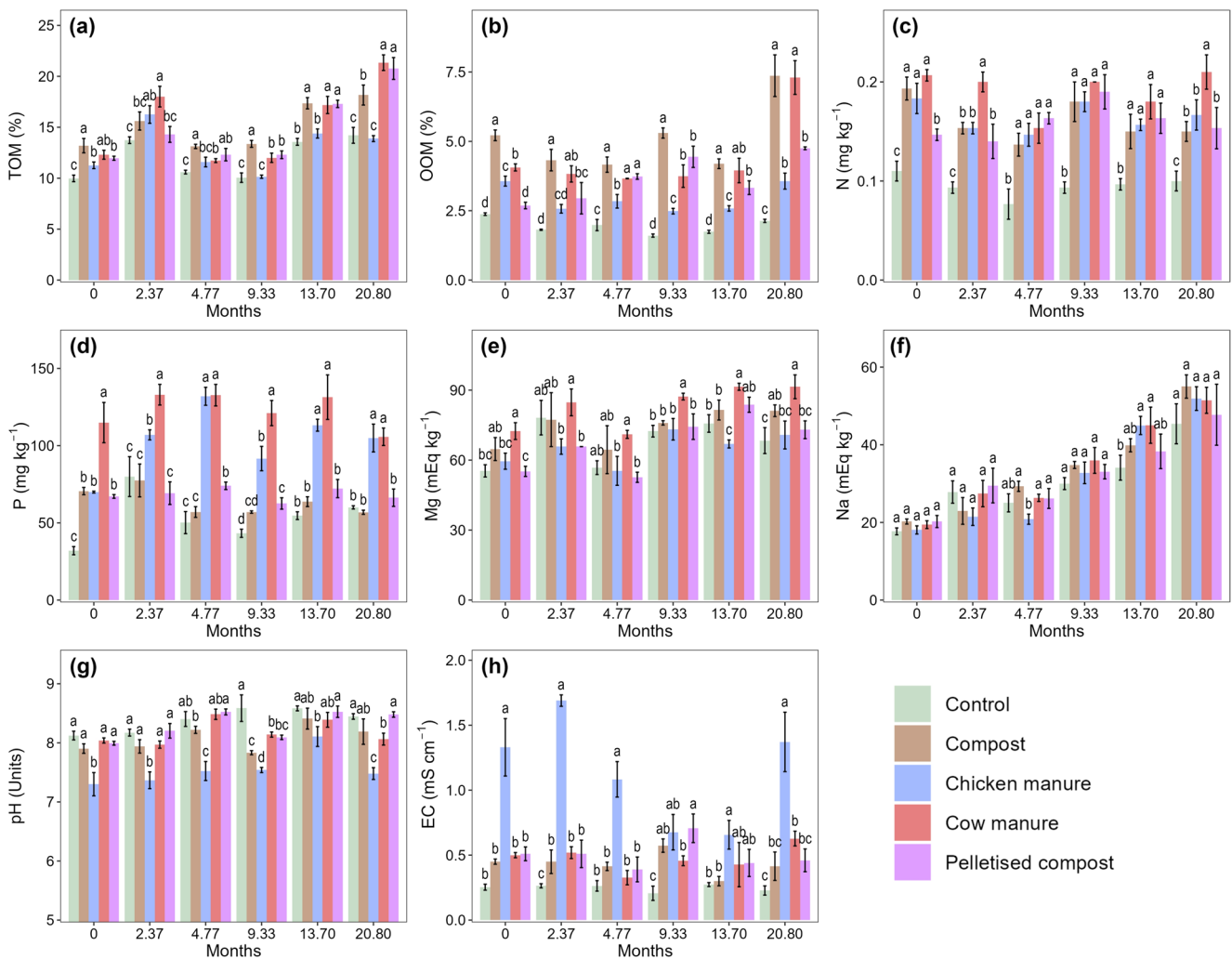


Fig. 1 Effect of different organic amendment treatments on soil chemical properties at the different sampling times. **(a):** Total Organic Matter (TOM), **(b):** Oxidisable Organic Matter (OOM), **(c):** Nitrogen (N), **(d):** Phosphorus (P), **(e):** Magnesium (Mg), **(f):** Sodium (Na), **(g):** pH

(aqueous extract), and **(h):** Electrical Conductivity (EC). Values are means \pm SD ($n=9$). Different letters indicate significant differences between treatments within each sampling time (Tukey, $p < 0.05$)

at most sampling times, especially at 2.37 and 20.80 months. While at 4.77, 9.33, and 13.70 months, no significant differences were found among amendments.

Cow and chicken manure significantly increased phosphorus content in the soil compared to the control and the other treatments throughout the experiment (except at time 0, where no significant difference was detected between chicken manure and the other amendments) (Fig. 1d). Likewise, at three sampling times (0, 2.37, and 9.33 months) the cow manure treatment showed a significantly higher phosphorus content than the chicken manure treatment. In contrast, no significant differences were detected between compost and pelletised compost treatments (except at 4.77 months), nor between these treatments and the control at 2.37, 13.70, and 20.80 months. Consequently, cow and chicken manure significantly enhanced soil phosphorus availability, whereas compost and pelletised compost provided only limited phosphorus input. Magnesium content in the soil increased with the addition of cow manure. At the 0, 9.33, 13.70 and 20.80 months sampling time, its level was significantly higher than the control treatment, and at the 9.33 months, it was also significantly higher than in the other amendments (Fig. 1e). The remaining treatments showed variability in their effects throughout the plant cycle, with no significant differences with the control treatment at several sampling times.

Soil sodium content was not significantly affected by the addition of OAs (Fig. 1f). This parameter progressively increased throughout the experiment in all treatments, ranging on average from 19.13 to 50.29 mEq kg⁻¹ between the beginning and the end of the assay.

Soil pH decreased significantly with the addition of chicken manure compared to the other treatments (Fig. 1g). Across all treatments, pH values ranged from 7.3 to 8.6 during the experiment. The mean pH for the chicken manure treatment across the six sampling times was 7.53, substantially lower than the means for the control (8.38), compost (8.08), cow manure (8.18), and pelletised compost (8.30). Nevertheless, at 13.70 months, no significant differences were observed between the chicken manure, compost, and cow manure treatments. Most other treatments did not differ significantly from each other or from the unamended control at most sampling times.

Electrical conductivity increased significantly with the addition of chicken manure compared to the control at all sampling times (Fig. 1h). At 0, 2.37, 4.77, and 20.80 months, EC of chicken manure was also significantly higher than all other amendments. At 9.33 months, it was significantly higher than the control only, and at 13.70 months, it was significantly higher than the control and compost treatment.

Furthermore, the effect of the addition of OAs on soil physicochemical parameters was analysed using PCA (Fig.

S2). Throughout the experiment, PC1 and PC2 together explained a large proportion of the total variance: PC1 consistently explained between 39.3% and 53.0%, while PC2 explained between 19.9% and 36.3% across all sampling times. The key variables influencing these components included TOM, OOM, nitrogen, pH and EC. The chicken manure (blue ellipse in Fig. S2) and cow manure (red ellipse) treatments were clearly distinguishable from the unamended control (green ellipse) in most cases, indicating a strong and persistent effect of these treatments on various soil parameters. In contrast, compost (brown ellipse) and pelletised compost (purple ellipse) showed a more moderate response, mainly associated with soil organic matter content. In summary, the amendment treatments differed markedly from the control treatment and their effects on the chemical properties of the soil system were maintained throughout the plant cycle.

3.2 Influence of Organic Amendments on Culturable Microbial Populations

The analysis of soil culturable microorganisms was performed using the pour plate method. Quantification of these microorganisms over time revealed that the different OAs significantly increased the abundance of culturable bacteria, actinobacteria, filamentous fungi, and *Trichoderma* spp. compared to the control treatment (Fig. 2).

Among the treatments, chicken manure caused the greatest increase in abundance of culturable bacterial populations, showing significantly higher cfu g⁻¹ counts than the control at all sampling times (Fig. 2a). In contrast, compost and pelletised compost had the lowest bacterial increases during the later stages (9.33, 13.70, and 20.80 months). Similar results were observed for culturable actinobacteria (Fig. 2b): chicken manure resulted in the highest cfu g⁻¹ of soil throughout the plant cycle and was significantly higher than the control and the pelletised compost treatments. Likewise, compost and cow manure amendments also led to significant increases compared to the control, however, at certain sampling times (4.77 and 9.33 months), their increases were not greater than those observed with the pelletised compost. In the case of culturable filamentous fungi, chicken manure caused the greatest increase, followed by compost (Fig. 2c). The fungal cfu g⁻¹ of the soil in the chicken manure treatment was significantly higher than the control from the beginning of the experiment until the 13.70 months sampling time, while the compost showed significant differences with the control treatment at 0, 2.37, 4.77, 9.33 and 20.80 months. The impact of OAs on *Trichoderma* populations varied among treatments (Fig. 2d). Cow manure had the highest counts, peaking at 933 cfu g⁻¹ at

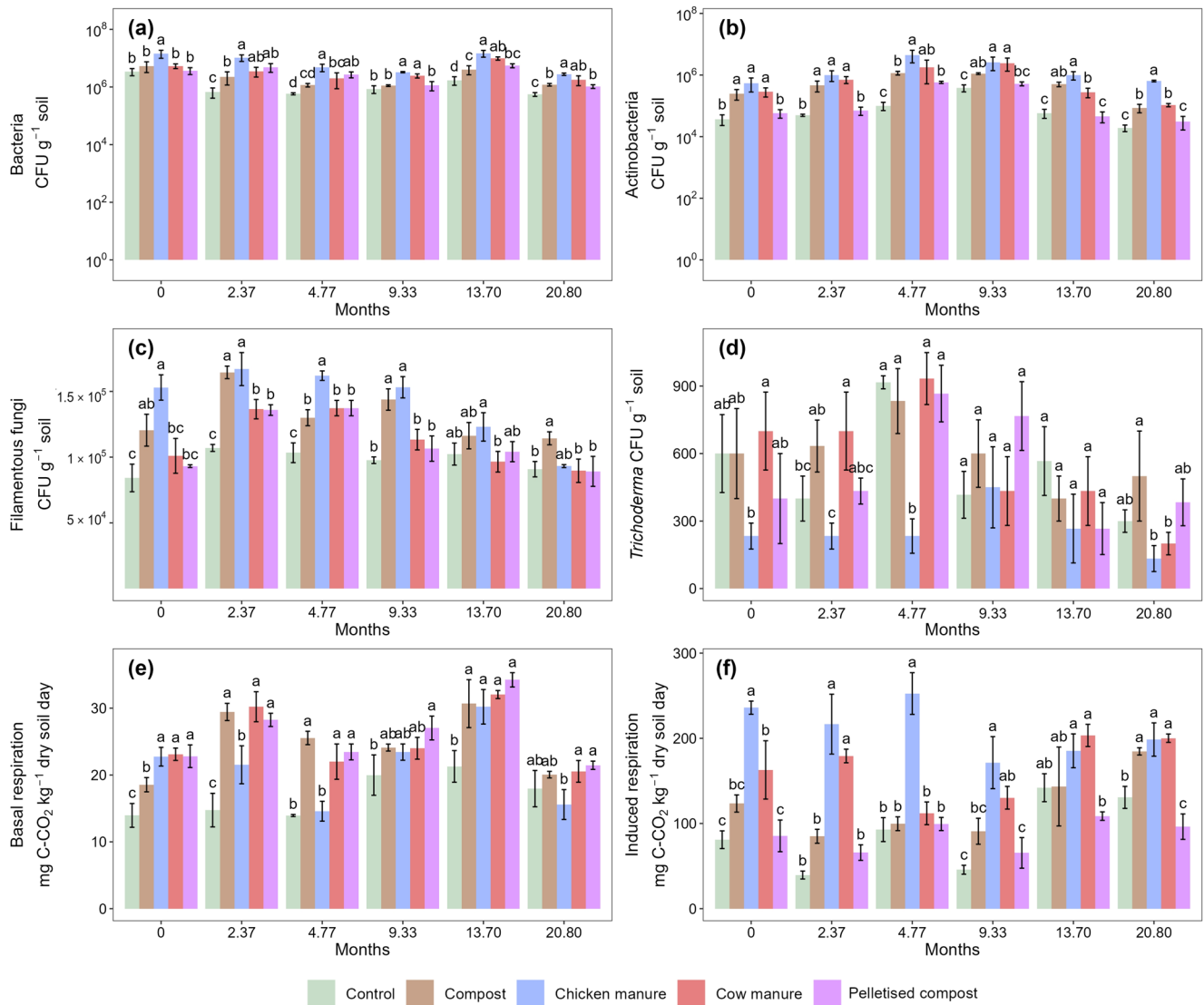


Fig. 2 Effect of different organic amendment treatments on microbiological parameters at the different sampling times. (a): Quantification of bacteria. (b): Quantification of actinobacteria. (c): Quantification of filamentous fungi. (d): Quantification of *Trichoderma* spp. (e): Basal

soil respiration. (f): Induced soil respiration. Values are means \pm SD ($n=9$). Different letters indicate significant differences between treatments within each sampling time (Tukey, $p < 0.05$)

4.77 months, significantly exceeding other treatments. The pelletised compost also showed high levels, reaching 866 cfu g^{-1} at 4.77 months, while chicken manure demonstrated lower and more variable counts throughout the experiment. Additionally, the influence of OAs on soil culturable microbial population composition was assessed through PCA (Fig. S3). The analysis revealed that while all amendments affected microbial dynamics, chicken manure (blue ellipse in Fig S3) had the most significant impact, with the remaining amendments showing less pronounced but still observable effects compared to the unamended control (green ellipse). Bacterial and actinobacteria populations were strongly linked and often clustered together from 4.77 months after, while fungal populations

tended to diverge, reflecting distinct responses under the influence of chicken manure. Overall, the PCA highlights the distinct role of chicken manure in shaping culturable microbial populations, particularly by enhancing bacterial and actinobacteria, while fungal populations exhibited a separate response pattern.

Basal and induced respiration measurements in amended soils revealed notable changes in microbial activity throughout the experiment. Basal respiration in amended treatments showed a significant increase compared to the control during the early stages (up to 4.77 months), except for the chicken manure treatment at 4.77 months (Fig. 2e). Subsequently, respiration values became more variable, with no treatment showing consistent dominance and, at

the end of the experiment, no significant differences were detected between the amended treatments and the control.

Induced respiration highlighted the early effects of chicken manure, which was significantly greater than the pelletised compost and the control at all sampling times, except at 13.70 months (Fig. 2f). However, at the end of the experiment (13.70 and 20.80 months) no significant differences were observed between the amendment treatments, with the exception of the pelletised compost.

To integrate and analyse the combined effects of the treatments on soil chemical and microbiological properties, a PCA was conducted (Fig. 3). The PCA results showed the distribution of samples treated with different OAs over time (0 to 20.80 months) along the first two principal components (PC1 and PC2), explaining 27.9% and 18.2% of the total variance, respectively. This analysis highlights the relationships between soil chemical, microbiological parameters and the OAs applied, helping to identify the most significant contributors to soil improvement. The control treatment (green ellipse in Fig. 3), located on the negative side of PC1, indicated lower nutrient levels and microbial activity in unamended soils. In contrast, the cow manure treatment (red ellipse), clustered on the positive side

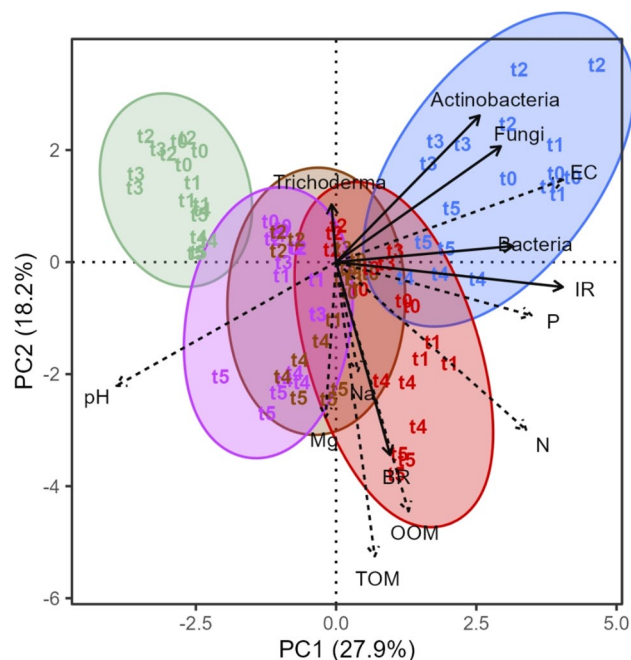


Fig. 3 Principal Component Analysis showing the effect of different organic amendments on soil properties over time (0 to 20.80 months). The ellipses represent the grouping of samples based on treatments: control (green), compost (brown), chicken manure (blue), cow manure (red), and pelletised compost (purple). Full arrows represent microbiological parameters and dashed arrows represent chemical soil parameters. EC: electrical conductivity; TOM: total organic matter; OOM: oxidisable organic matter; N: nitrogen; P: phosphorus; Mg: magnesium; Na: sodium; pH: pH in aqueous extract, BR: basal respiration, IR: induced respiration

of PC1, strongly associated with variables such as TOM, OOM, basal respiration, and nitrogen. This suggests that cow manure consistently and significantly enhanced soil fertility by increasing nutrient availability (especially nitrogen) and stimulating microbial activity, as reflected in higher basal respiration values. The chicken manure treatment (blue ellipse) was predominantly located along the positive axis of PC2, showing a strong correlation with bacterial, actinobacterial, and filamentous fungi counts, electrical conductivity, and induced respiration. This distribution indicates that chicken manure primarily enhanced biological activity and microbial populations in the soil. However, compared to cow manure, the effect of chicken manure is more focused on biological and conductivity-related parameters rather than improving soil fertility indicators such as organic matter or nutrient content. The pelletised compost (Fig. 3, purple ellipse) and the compost treatments (brown ellipse) exhibited broader dispersions across both principal components, indicating more variable responses in terms of nutrient availability and microbial activity. Compost treatments aligned with the positive side of PC1, especially at later sampling times, suggesting moderate increases in soil fertility (e.g., TOM and OOM content). Additionally, related to microbial populations, *Trichoderma* populations were more associated with cow manure and compost treatments, indicating that these amendments could promote the growth of this potential biocontrol fungus throughout the experiment. Overall, the PCA clearly illustrates the distinct effects of OAs on soil properties, highlighting cow and chicken manure as the most impactful treatments in terms of soil nutrient content and biological activity throughout the course of this experiment.

3.3 Effect of Organic Amendments on Banana Plant Development and Nutrient Uptake

The application of OAs resulted in variable effects on plant biometric parameters. Chicken and cow manure treatments were the most effective, significantly increasing total height, weight, aerial and root fresh weight, pseudostem circumference, leaf number, and leaf area (Fig. 4 and S4). For instance, plants amended with chicken and cow manure reached average heights of 240 cm and 227 cm, respectively, significantly higher than the control and other treatments (Fig. 4a). The number of leaves and leaf area were significantly greater in all amended treatments compared to the control, with chicken and cow manure yielding the highest values (Fig. 4b-c). Similarly, these treatments resulted in significantly greater pseudostem circumference compared to the other treatments (Fig. 4d). In terms of weights (Fig. 4e-g), cow and chicken manure were the most effective treatments for increasing aerial fresh weight, showing significantly

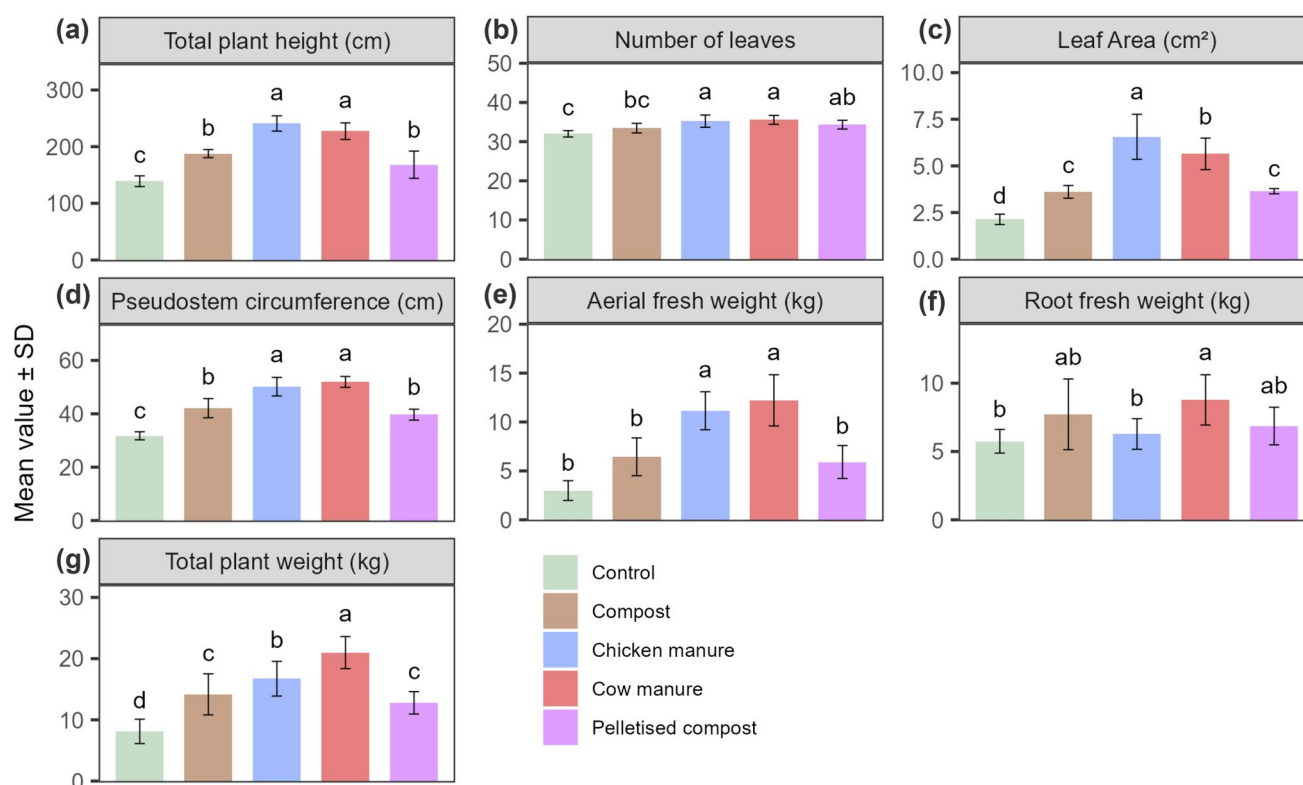


Fig. 4 Effect of different organic amendment treatments on biometrics parameters on plant at the final sampling time (20.88 months). The soil was amended with chicken manure, cow manure, compost, and pelletised compost. A treatment without inputs was used as control. (a): Total plant height. (b): Number of leaves. (c): Leaf area. (d): Pseu-

dostem circumference. (e): Aerial fresh weight. (f): Root fresh weight. (g): Total plant weight. Values are means \pm SD ($n=9$). Different letters above the error bars indicate significant differences between treatments in each parameter (Tukey, $p < 0.05$)

higher values than the other treatments. In contrast, compost and pelletised compost did not differ from the control. Notably, root weight in the chicken manure treatment was not significantly different from the control (Fig. 4f), while other amendments increased this parameter. Overall, total plant weight was significantly improved by all OAs, with cow manure producing the highest value (21 kg; Fig. 4g).

In terms of yield, only the plants treated with chicken and cow manure produced fruit (Table 1). Chicken manure significantly outperformed cow manure in several yield parameters, including higher bunch weight (7.97 vs. 3.50 kg), hand weights (1.27 vs. 0.49 kg), number of fingers per hand (10.65 vs. 9.50), finger diameter (3.88 vs. 2.46 cm), and rachis (flower stalk) diameters (4.16 vs. 3.40 cm). However, no significant differences were observed between the treatments in terms of rachis weight, bunch length, number of hands per bunch, or finger length. Overall, chicken manure was the most effective treatment, outperforming cow manure in yield quantity and fruit quality across several parameters.

Regarding plant tissue nutrition at harvest (20.80 months), chicken manure was the most effective treatment for nutrient uptake (Fig. 5). It significantly increased calcium

(23.18 g kg⁻¹), iron (77.07 mg kg⁻¹), manganese (184.24 mg kg⁻¹), nitrogen (22.88 g kg⁻¹), potassium (40.20 g kg⁻¹), sodium (0.40 g kg⁻¹), and zinc (28.70 mg kg⁻¹) contents compared to the other treatments. However, no differences

Table 1 Effect of chicken and cow manure treatments on banana plant yields

	Chicken manure	Cow manure	sig
Yield ($N^T=9$)	9	5	
Bunch Weight (kg)	7.97 \pm 1.43	3.50 \pm 0.81	***
Bunch Length (cm)	99.44 \pm 12.38	95.20 \pm 19.38	ns
Hands per bunch	5.77 \pm 0.66	4.80 \pm 0.83	ns
Weight of hands (kg)	1.27 \pm 0.43	0.49 \pm 0.20	***
Number of fingers	10.65 \pm 2.17	9.5 \pm 0.97	**
Length of finger (cm)	18.88 \pm 3.06	16.83 \pm 2.00	ns
Diameter of finger (cm)	3.88 \pm 3.29	2.46 \pm 0.34	**
Diameter of rachis (cm)	4.16 \pm 0.13	3.40 \pm 0.21	***
Weight of rachis (kg)	0.80 \pm 0.30	1.21 \pm 0.29	ns

Values represent mean \pm standard deviation ($n=9$). Different asterisks in the right column indicate the significance level of the differences between chicken and cow manure treatments (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ns: no significant, T-test). The other treatments are not included as they did not result in yield production during the experiment

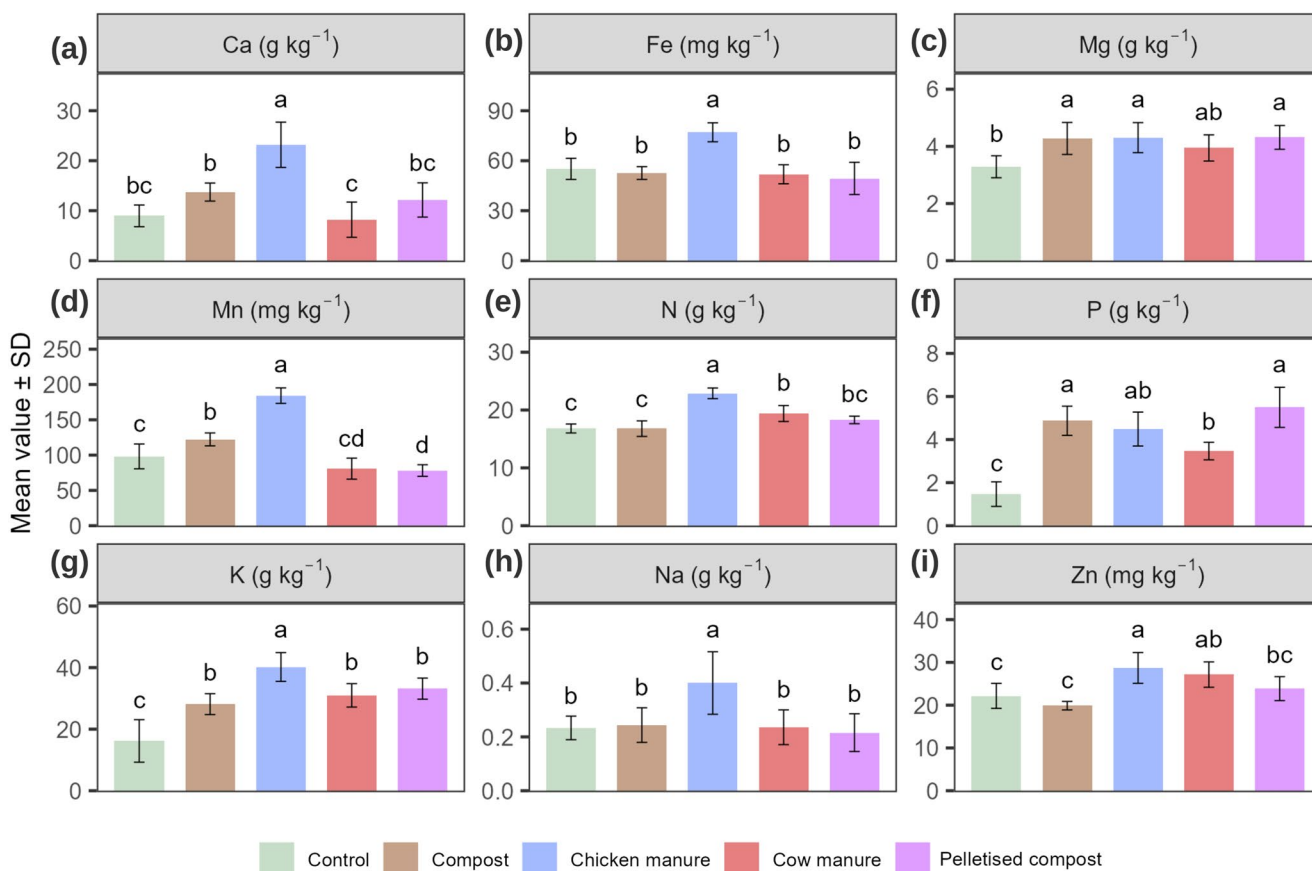


Fig. 5 Effect of different organic amendment treatments on leaf nutrient content at the final sampling time (20.88 months). The soil was amended with chicken manure, cow manure, compost, and pelletised compost. A treatment without inputs was used as control. Ca: calcium.

Fe: iron. Mg: magnesium. Mn: manganese. N: nitrogen. P: phosphorus. K: potassium. Na: sodium. Zn: zinc. Values are means \pm SD ($n=9$). Different letters above the error bars indicate significant differences between treatments in each parameter (Tukey, $p < 0.05$)

were observed between amendments in magnesium and phosphorus (except cow manure) plant content.

4 Discussion

The application of OAs had a significant impact on the soil chemical properties over the course of this experiment. Across the entire study, OAs contributed to substantial improvements in soil quality, as evidenced by increased levels of TOM, OOM, and essential nutrients such as nitrogen, phosphorus, and magnesium (Fig. 1a-e). These findings are consistent with previous studies showing that OAs, including compost and manure, enhance soil organic carbon (an indicator of soil health) and nutrient levels in banana crops (Adriano et al. 2012; Bass et al. 2016; Mazinga Kwey et al. 2021; Sunarta and Trigunasih 2017; Zhang et al. 2019, 2020).

Among the amendments tested, compost exhibited the highest OOM values throughout the experiment (5.09% on average), followed by cow manure (4.42%), pelletised

compost (3.65%) and chicken manure (2.94%) (Fig. 1b). Similar results have been observed in previous research; for instance, Sunarta and Trigunasih (2017) reported a greater increase in organic carbon with cow manure than with chicken manure, while Bass et al. (2016) also emphasized the consistent positive effect of compost and biochar on soil organic carbon. In our study, soil organic matter progressively increased over time, reaching its highest levels at the end of the plant cycle. This pattern can be attributed to the cumulative effect of both the organic amendments and the root biological activity, which is a key factor in the buildup of soil organic matter. Plant roots release carbon into the soil through exudation, while decomposing roots further contribute organic matter, as reported by different studies (Koishi et al. 2020; Leroy et al. 2008; Mekki et al. 2019; Pan and Huang 2024).

Phosphorus levels in the soil significantly increased with the addition of cow and chicken manure (Fig. 1d), which is consistent with the findings of Ara et al. (2018) and Nguyen et al. (2021). The high phosphorus content of these OAs promoted its mineralization and enhanced phosphorus

availability in the soil, which is essential for plant growth. In contrast, compost and pelletized compost had a less pronounced effect on phosphorus availability, likely due to their lower phosphorus content (Table S2) and probably slower mineralization rate.

In terms of soil pH, chicken manure was the only amendment that caused a significant decrease compared to the other treatments. This reduction is consistent with findings by Zhang et al. (2020), who observed pH declines with certain OAs, particularly those with lower pH or higher acidifying potential. For most other amendments, however, pH remained stable, likely due to the buffering capacity of organic matter in the soil. Similarly, Jayalath et al. (2016) observed pH reductions with some OAs, while Toncea et al. (2015) noted that long-term organic management with plant residues can lead to soil acidification, especially in continuous organic cultivation systems.

Soil sodium levels increased throughout the experiment; however, this did not result in a significant rise in electrical conductivity, suggesting that the organic amendments may have buffered the effects of increased salinity. The irrigation water used in this study had an alkaline pH (8.6) and a relatively high sodium concentration (7.4 mEq L^{-1}), which likely contributed to the gradual increase in soil sodium levels (Fig. 1f). Interestingly, although the pellet amendment contained the highest sodium concentration ($237.10 \text{ mEq kg}^{-1}$) (Table S2), no significant differences in sodium levels were found among treatments in the soil, either at the beginning or at the end of the experiment (Fig. 1f). This suggests that, despite the higher sodium content in the pellet amendment, its impact on soil sodium levels was buffered, potentially by other factors such as cation exchange capacity and the organic matter content of the amendments. Organic amendments have been reported to improve the physicochemical properties of saline-alkali soils, such as increasing cation exchange capacity and reducing salt content (Hoque et al. 2022; Irin and Hasanuzzaman 2024a,b), potentially mitigating the impact of sodium-rich irrigation water on salinity accumulation. Although chicken manure exhibited relatively high electrical conductivity compared to other treatments, the electrical conductivity values remained below the phytotoxicity threshold for bananas, indicating no evidence of salinity stress. These findings suggest that organic amendments can mitigate salinity stress by enhancing water and nutrient uptake by plant roots (Hoque et al. 2022; Li et al. 2022). Moreover, the effects of sodium and electrical conductivity on soil properties and plant performance are influenced by several factors, including the specific amendment used, soil composition, and water quality, highlighting the need for tailored management strategies in banana agroecosystems (Bass et al. 2016; Irin and Hasanuzzaman 2024a; Sunarta and Trigunasih 2017).

Soil microorganisms play a crucial role in maintaining soil health, and the application of OAs is known to significantly influence their abundance, diversity, and activity. The addition of organic matter is widely recognised for enhancing soil physicochemical properties and promoting microbial functionality (Liu et al. 2017; Mäder et al. 2002). However, these effects strongly depend on the type and characteristics of the OAs applied. For instance, easily degradable materials (generally with low C/N ratio) typically support fast-growing copiotrophic organisms in nutrient-rich environments, whereas more resistant materials, such as straw, promote cellulolytic microorganisms specialized in breaking down cellulose (Hu et al. 1999; Jensen and Nybroe 1999; Chou et al. 2017). Therefore, the addition of OAs to the soil has a significant impact on microbial composition, influenced by the quality and composition of the applied amendments (Liu et al. 2023).

In our study, the different types of OAs, such as chicken manure, cow manure, compost, and pelletized compost, showed variations in their effects on culturable microbial populations, which were related to their nutrient content and chemical characteristics (Table S2). Chicken manure, with a low C/N ratio (11.59) and high nitrogen content (3.47%), significantly promoted the proliferation of bacteria, actinobacteria, and filamentous fungi in the soil, which was reflected in a considerable increase in these microorganisms during most of the plant cycle. Consequently, the low C/N ratio allowed for rapid decomposition of organic material and facilitated nutrient availability, which is a key factor for microbial development. Moreover, the high content of phosphorus ($979.20 \text{ mg kg}^{-1}$), calcium ($831.00 \text{ mEq kg}^{-1}$), magnesium ($200.80 \text{ mEq kg}^{-1}$), and potassium ($482.60 \text{ mEq kg}^{-1}$) in chicken manure likely also contributed to significantly increasing microbial activity and soil fertility compared to the control treatment. These results are consistent with previous studies that have found that different manures, rich in soluble nutrients, are very effective in improving microbial activity in agricultural soils (Liu et al. 2023; Li et al. 2020). In contrast, cow manure, with a higher C/N ratio (19.14) and lower nitrogen content (2.06%) due to the presence of straw, mainly favored actinobacterial populations, which are effective in decomposing more complex and recalcitrant compounds, such as cellulose. The high phosphorus content ($935.00 \text{ mg kg}^{-1}$) may also have contributed to enhanced microbial activity. Therefore, cow manure provided a more stable and slower-releasing substrate for microorganisms, resulting in a more sustained microbial impact over time, favoring the proliferation of actinobacteria and other microorganisms specialized in decomposing more stable materials. On the other hand, both compost and pelletized compost showed more moderate effects on microbial abundance, which can be explained by

their higher C/N ratio (compost: 24.05, pelletized: 21.83), indicating that these materials release nutrients more gradually, thus affecting microbial dynamics over the long term. However, these treatments, along with cow manure, showed increases in *Trichoderma* spp. populations, which are known for their biocontrol ability against soil pathogens, such as *Fusarium oxysporum* (Heck et al. 2019). This is particularly relevant in the Canary Islands, where banana crops are affected by the Subtropical Race 4 of *Fusarium oxysporum* f. sp. *cubense* (Perera et al. 2024). Consequently, the different amendments led to significant increases in the abundance of culturable populations of mesophilic aerobic bacteria, actinobacteria, and filamentous fungi. Similar findings have been reported in banana systems, where the use of bio-organic and compost-based fertilizers enhanced bacterial, actinobacterial, and fungal populations, improving microbial diversity and reducing the incidence of soil-borne diseases such as *Fusarium* wilt (Shen et al. 2015; Zhang et al. 2019; De Corato 2020). Furthermore, repeated applications of organic matter, rather than a single dose, have been shown to be more effective in maintaining microbial activity and nutrient cycling over time (Marschner et al. 2003).

In terms of microbial activity, basal and induced respiration methods were used to evaluate the effects of the amendments on soil microbiological functioning (Fig. 2e-f). On average, amendments increased basal respiration by 1.5 times compared to the control across the plant cycle, with pelletised compost showing the highest increase (1.7 times) and chicken manure the lowest (1.2 times) (Fig. 2e). Similarly, induced respiration also increased by an average of 1.5 times compared to the control (Fig. 2f), with chicken manure showing the most significant increase (2.4 times), while pelletised compost had the smallest increase at the end of the experiment. These results are closely related to the C/N ratio of each amendment, with the greatest response to glucose addition (induced respiration) observed in the amendment with the lowest C/N ratio (chicken manure). In contrast, the higher C/N ratios of cow manure (19.14), compost (24.05), and pelletized compost (21.83) resulted in a lower response in induced respiration. This highlights the fundamental role of the C/N ratio in influencing microbial metabolism and carbon availability in the soil. The literature also supports the positive influence of organic inputs on microbial respiration across different environments, including banana agrosystems. For example, Durango Cabanilla (2017) reported substantial increases in microbial respiration following the application of vinasse and compost, while Bass et al. (2016) observed elevated CO₂ emissions in banana soils treated with compost and biochar, attributing this to enhanced availability of labile carbon for microbial metabolism. These findings are consistent

with our results, where both basal and induced respiration increased markedly in amended soils (Fig. 2e-f).

Consequently, the chemical composition of organic amendments plays a key role in soil microbial dynamics. Chicken manure and cow manure were the most effective treatments for increasing the culturable microbial populations, due to their high nutrient availability and capacity to favor different microbial groups. However, compost and pelletized compost showed more moderate effects. Therefore, these results highlight the importance of selecting the appropriate amendment based on specific soil management objectives, whether for enhancing microbial activity, fertility, or disease control.

The impact of organic amendments on plant development and nutrient uptake is fundamental for enhancing crop productivity. In this study, the addition of OAs significantly enhanced plant growth, with fruit production observed only in the chicken and cow manure treatments (Fig. 4, Table 1). These findings are in agreement with previous research demonstrating the positive impact of organic fertilizers on plant growth and productivity. For instance, Zhang et al. (2019) reported significant increases in green leaf counts, plant height, pseudostem circumference, and leaf area at harvest when organic fertilizers were applied. Similarly, EL-Gioushy et al. (2022) observed substantial improvements in growth and fruit yield in banana plants under organic fertilization. In our study, both chicken and cow manure treatments significantly improved biometric parameters such as total plant height, leaf area, pseudostem circumference, aerial fresh weight, and total plant weight.

In terms of yield, cow and chicken manures treatments led to significant increases in finger number, diameter, and overall fruit size and weight (Table 1). These results are consistent with Rahman et al. (2021), who reported that the combined use of farmyard manure and microbial consortia increased bunch weight and fruit size in banana plants. Similarly, Durango Cabanilla (2017) reported improvements in yield and fruit size after applying compost to banana crops. Several mechanisms may explain the increased yield observed in the organic amendment treatments. Organic inputs improve soil structure, aeration, and water-holding capacity, favouring root growth and nutrient uptake (Blanco-Canqui et al. 2024). Manure applications also enhance soil quality by increasing cation exchange capacity, which improves the retention of essential cations such as potassium, calcium, and magnesium, critical for plant growth. Furthermore, organic amendments increase microbial activity in the soil, which plays a crucial role in nutrient cycling and availability. In fact, a meta-analysis by Du et al. (2020) showed that manure applications significantly increased crop yields by 7.6% compared to mineral fertilizers, with even greater productivity increases over

longer periods. This increase was attributed to improvements in key soil properties such as pH, soil organic carbon, and nutrient availability, alongside increased microbial abundance and enzymatic activity.

To evaluate the nutritional status of banana plants, we analysed the effects of OAs on both macronutrient and micronutrient content. All amendments significantly affected nutrient concentrations, with chicken and cow manure treatments showing the most pronounced effects (Fig. 5). Notably, chicken manure significantly increased the concentrations of nitrogen, calcium, potassium, iron, manganese, sodium, and zinc, demonstrating the greatest effect on multiple nutrients. To better understand the implications of these findings, we compared the foliar nutrient concentrations at harvest with the optimal nutrient ranges for banana crops (Alvarez et al. 2001; BOC 2003; Pérez Almedia 2003). Overall, nitrogen, potassium, and calcium levels in plants receiving organic amendments approached or reached the recommended ranges for optimal banana growth, particularly in the chicken and cow manure treatments, whereas the control plants remained below the optimal nutritional status. Although nitrogen levels did not fully reach the recommended optimal range, plants under organic amendments showed improved nutrient uptake compared to the control. These findings indicate that organic amendments, especially livestock manures, were effective in enhancing leaf nutritional status, even in the absence of chemical fertilization. Consistent with this, previous research has shown that organic amendments, especially chicken manure, can significantly enhance nitrogen, potassium, and calcium levels in banana crops (Mazinga Kwey et al. 2021). Similarly, Selvamani and Manivannan (2009) observed higher nitrogen and potassium levels in banana leaves treated with organic manures, particularly farmyard manure and vermicompost. In contrast, Zhang et al. (2020) reported no significant differences in potassium, phosphorus, or calcium levels in banana plants treated with organic fertilizers like plant residues. However, our results showed that all amendments significantly increased the potassium and phosphorus content in leaves compared to the control, while chicken manure significantly increased calcium levels. These results underscore the importance of the type and composition of organic amendments, which influence soil nutrient dynamics, microbial activity, nutrient solubility, and ultimately nutrient uptake by the plants.

Finally, while the positive effects of organic amendments on soil health and plant productivity are evident, it is also important to recognize the potential trade-offs associated with their use. Although organic amendments, such as

manure and compost, can significantly improve soil structure, nutrient availability, and microbial activity, they may also introduce risks related to soil contamination and human health. The application of organic amendments to agricultural soils can result in the accumulation of heavy metals, pathogens, antibiotic residues, and other emerging contaminants (Goss et al. 2013; Urra et al. 2019). These substances can pose significant challenges to both environmental and human health, as they may disrupt soil microbial communities and contaminate food and water resources. Therefore, it is essential to balance the benefits of organic amendments with the potential risks they present. Strategies such as the proper treatment of amendments prior to application and continuous monitoring of their environmental impact are crucial to mitigate these risks.

5 Conclusion

This study demonstrates the significant effects of organic amendments commonly used in banana crops in the Canary Islands (cow manure, chicken manure, compost, and pelletised compost) on soil health, plant growth, and productivity. The findings support the hypothesis that organic amendments improve soil fertility by enhancing microbial activity, soil chemical properties, and nutrient cycling, with distinct effects depending on the type of amendment applied. Therefore, organic amendments, particularly cow and chicken manure, offer a sustainable alternative to chemical fertilizers for banana production. The observed improvements in soil microbial activity and nutrient cycling highlight their potential to enhance soil health and productivity. However, the study also emphasizes the need for long-term research to address challenges such as pathogen presence, nutrient imbalances and heavy metal accumulation, which could limit the long-term benefits of organic amendments. Additionally, repeated applications and different combinations of these amendments across multiple production cycles may reveal synergistic effects that further enhance soil health and plant productivity. Incorporating molecular tools, such as metabarcoding, will be essential to investigate the influence of organic amendments on microbial communities and to understand their potential in managing soil-borne diseases, such as *Fusarium* wilt, which remains a significant challenge in banana production systems.

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Data Availability The data of this study are available from the corresponding author upon request.

Code Availability Not applicable.

Declarations

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication All authors have read and agreed to the published version of the manuscript.

Conflict of interest The authors declare that they have no conflict of interest.

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