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Interaction between *Trichoderma* sp., *Pseudomonas putida*, and Two Organic Amendments on the Yield and Quality of Strawberries (*Fragaria x annanasa* cv. San Andreas) in the Huaral Region, Peru

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Abstract: Strawberry cultivation holds significant economic and social promise within Peruvian fruit production. However, conventional management practices have led to the excessive use of agrochemicals in this crop. This study proposes an organic approach to strawberry production, integrating less environmentally harmful technologies. The aim was to assess microbial inoculation by using *Trichoderma* sp. and *Pseudomonas putida* and the application of organic amendments on strawberry seedlings of the commercial cultivar "San Andreas". A field experiment was established with evaluations in the vegetative and productive stages. Results indicate that the co-inoculation of *Trichoderma* sp. and *Pseudomonas putida* increased leaf area by 7%, and enhanced the aerial part's fresh and dry biomass by 13% and 28%, respectively, compared to treatment without microbial inoculation. Concurrently, compost application increased the leaf number and aerial dry biomass by 22% and 19% at the end of the vegetative stage, respectively, compared to treatment without organic amendment. In addition, it reduced the days for flowering, maintaining the fruit's physicochemical attributes. Regarding yield, the amendments application significantly enhanced fruit weight per plant by 40%, especially when applied together with *Trichoderma* sp., and co-inoculation increased the number of fruits per meter square by 22%. These findings highlight the potential of technologies such as microbial inoculation and organic amendments to enhance strawberry yields and to gradually reduce the use of synthetic fertilizers.

Keywords: *Fragaria* sp.; microbial inoculants; compost; leaf litter; *Trichoderma* sp.; *Pseudomonas putida*

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1. Introduction

Strawberry (*Fragaria* sp.) is one of the most appreciated cultivated berries in the world due to its high economic and nutritional value [1]. In 2020, global strawberry production value reached USD 14 billion [2]. Moreover, strawberry consumption provides essential nutrients and high vitamin C and folate levels. In addition, strawberries are rich in bioactive compounds such as phenolic compounds, which together with vitamin C act as antioxidants in the human diet [3].

In 2022, more than 9.5 million tons were produced globally, with China and the United States leading production [2]. Latin America contributed 5% of the total production, with

Peru being the third largest producer with almost 46,000 tons of volume [2]. Peruvian production value doubled during 2020–2022. Sowed areas are concentrated in the coastal regions, mainly in Lima and La Libertad, but in recent years cultivation has expanded to the Andes regions or inter-Andean valleys [4]. In addition, strawberry cultivation generates a considerable labor demand for harvesting, thus providing work opportunities throughout the year.

Most strawberry fields in Peru are managed by small producers, typically cultivating areas of no more than three hectares [4], and they often receive minimal technical and financial assistance [5]. The main source of information that strawberry growers have regarding chemical input use is through sales personnel in commercial houses [6], which in many cases causes the excessive use of agrochemicals. This global problem has negatively impacted the environment, leading to ecosystem degradation. In 2018, total global emissions from agriculture and related land use reached 9.3 billion tons of equivalent CO_2 [7], which contributes the most to the greenhouse effect. N_2O emissions are expected to increase between 30–60% until 2030 [8] and fertilizer consumption by developing countries will exceed 75% of world consumption in 2050 [9]. Furthermore, the excessive use of fertilizers has led to a significant reduction in the economic efficiency of strawberry production [10].

In this context, bio-input use emerges as an alternative to generate cleaner and more sustainable production systems [11]. Among practices associated with bio-input use, the incorporation of plant-growth-promoting microorganisms into the soil can provide nutrients to plants, stimulate their growth, or protect them from pathogen attack [12]. Thus, microbial inoculation reduces the need for fertilizers, an increasingly important strategy being adopted worldwide. *Trichoderma* is a genus widely known for its antagonistic capacity; however, some strains of *Trichoderma* spp. have beneficial effects on plant growth [13]. These strains operate through various mechanisms, including the biological control of phytopathogens, improvement in nutrient absorption, increased root hair formation, and the induction of systemic resistance in plants [14]. Likewise, *Pseudomonas* species influence plant growth due to their ability to produce siderophores, solubilizing phosphorus and secreting antagonistic compounds for plant pathogens [15].

Solid waste management through organic composting is also a potential alternative for agricultural system sustainability. Organic amendments are used to improve the physical, microbiological, and chemical conditions of soils thereby increasing plant nutrient availability [16]. Thus, compost is an excellent organic fertilizer because it provides essential nutrients and organic matter. Additionally, compost enhances the soil's water retention capacity, improves tillage, promotes better aeration for seed germination, and consequently supports robust plant root development [17].

Undoubtedly, the strawberry is a crop with great export potential and profitability in Peru [18]. Still, there is a need to explore new technologies that are environmentally friendly to our soil integrity and human health. Therefore, the objective of this study is to evaluate the influence of microbial inoculation and the application of organic amendments on strawberry (*Fragaria x ananassa* cv. San Andreas) yield and quality, as organic alternatives for a more sustainable management of this crop.

2. Materials and Methods

2.1. Plant Material and Sowing

Strawberry plants (*Fragaria* sp. var. San Andreas) were obtained from the National Vegetable Research Program (PNIH) of the National Institute for Agrarian Innovation (INIA), as 3-month-old bare-root seedlings. Field installation was carried out in June 2023 at the Donoso Agricultural Experimental Station of INIA in Huaral Valley, and the experiment lasted seven months, from transplanting to harvest. The Huaral region is influenced by winter fogs and drizzles, creating a cool and humid environment in the middle of the desert. During the period from June to December 2023, the average temperature was 19.5 °C (max. 23.3 °C, min. 17.0 °C) and the relative humidity was 80.5%. The experimental area was 172.8 m^2. The soil of the experimental plot was sandy clay–loam in texture, low in organic matter (0.7%), with 8.0 soil pH; the available N, P, and K were 15, 109, and 150 kg ha^{-1}, respectively. The planting frame during transplanting was 0.8 m between furrows and 0.2 m between plants, with a total density of 62,500 plants per hectare. The field was irrigated 1–2 times per week, according to demand, and manual weeding was carried out every two weeks. No synthetic fertilizers or agrochemicals (for pest control) were used during the experimental area management.

2.2. Design and Treatments

A Completely Randomized Block Design (CRBD) experimental plot was set up with a 4 × 3 factorial scheme, involving four combinations of microorganisms and three organic amendments. A total of 12 treatments were used and considering that 3 blocks were installed in the plot, a total of 36 experimental units were managed during the whole research. The different treatments used in the experiment are described in Table 1.

Table 1. Evaluated factors and treatments.

Treat.	Factor 1. Microbial Inoculation	Factor 2. Organic Amendments Application
T1	Without microbial inoculation (WM)	Without amendments (WA)
T2		Compost (C)
T3		Manure + Leaf litter (M)
T4	*Trichoderma viride* (T)	Without amendments (WA)
T5		Compost (C)
T6		Manure + Leaf litter (M)
T7	*Pseudomonas putida* (P)	Without amendments (WA)
T8		Compost (C)
T9		Manure + Leaf litter (M)
T10	*Trichoderma viride* + *Pseudomonas putida* (T + P)	Without amendments (WA)
T11		Compost (C)
T12		Manure + Leaf litter (M)

The organic amendments application (compost and cow manure + avocado stubble) was carried out five days before sowing, at a 15 t ha^{-1} for compost, 7.5 t ha^{-1} for manure, and 7.5 t ha^{-1} for avocado stubble dose. The pH of the organic amendments was around 8.0 and the electrical conductivity was low for the manure and compost (<1 $dS \cdot m^{-1}$) but high for the leaf litter (2.2 $dS \cdot m^{-1}$). The C:N ratios for the compost, manure, and leaf litter were 10.0, 19.8, and 26.8, respectively. Microbial inoculation was carried out 4 times during the trial; the first at sowing and the remaining as complementary inoculations in the field at 21, 50, and 77 days after transplanting (dat). The first inoculation consisted of immersing the roots inside the inoculant for 15 min, and then transplanting the seedlings in the field [19], ensuring that the crown was at ground level. Complementary inoculations were carried out by adding 2 L of the inoculant to each plant's neck [20].

2.3. Inoculant Preparation

For inoculation with *Trichoderma* sp., a spore suspension obtained by washing sporulated broken maize with this fungus (Soluciones Agrosostenibles S.A.C., Trujillo, Peru, 1×10^{12} conidia per kg concentration,) in non-chlorinated water at 40 $g \cdot L^{-1}$ was prepared (Solution A, inoculant). For inoculation with *Pseudomonas putida*, a pure culture of *P. putida* strain PS168 (private collection) was prepared, from which a 1% (v/v) dilution was made for the inoculation. Pure culture was prepared by incubating the strain in nutrient broth for 4 days at 28 °C until a 10^9 CFU mL^{-1} concentration (Solution B) was obtained. Subsequently, Solution B was diluted by taking a 10 mL aliquot and mixing it with 1 L of non-chlorinated water (Solution C, inoculant). For treatments containing both microorganisms, the inoculant was prepared at a rate of 10 mL of pure culture of *Pseudomonas putida* (Solution B) for each 1 L of spore suspension (Solution A).

2.4. Biometric Parameters

The seedling mortality percentage was assessed at 30 dat. In addition, on five randomly marked plants per treatment, the leaf number, plant height (cm), and leaf area (cm^2) were assessed monthly until 90 dat. The leaf number was assessed by manually counting the leaves on each plant; the height was measured from the plant's crown to the last point of plant biomass, without spreading the leaves; and the leaf area was calculated by measuring the width and length of each leaf on the plant. Three plants were randomly removed to assess the fresh and dry biomass at 90 and 160 dat. For this, the root part was separated from the aerial part and the fresh weight of each one was recorded by a high precision balance (Axis Aka 4200, Gdañsk, Poland). They were then placed in paper bags and dried initially at room temperature for three weeks, followed by further drying in a drying oven (Yamato Scientific DS-64, Santa Clara, CA, USA) at 70 °C for 3 days [21].

2.5. Performance and Quality Parameters

From 90 dat onwards, plants were evaluated daily to observe the appearance of the first flower in each treatment, thereby calculating the number of days to flowering. In addition, in five randomly marked plants in each experimental unit, the number of flowers, the fresh weight (g), and the fruit dimensions (cm) were evaluated weekly. Additionally, the number of commercially ripe fruits (minimum 75% of the total fruit surface with red coloring) per experimental unit was recorded. At 160 dat, the fruits were harvested. The cell juice pH was evaluated using a multi-parameter meter (Hanna HI2020, Hanna Instruments, Leighton Buzzard, Bedfordshire, UK) by the AOAC 981.12 method; the acidity by titration with 0.1 N sodium hydroxide (AOAC 942.15 method); the Brix degrees by using a digital refractometer (PAL-1 Atago 3810, ATAGO, Tokyo, Japan); and the firmness by using a penetrometer (Lutron FR-5120, Lutron, New York City, NY, USA).

2.6. Statistical Analysis

The results were analyzed using R software version 4.3.1 (Lucent Technologies, Murray Hill, NJ, USA), employing the analysis of variance (ANOVA) with a significance level of 0.05, following verification of data normality assumptions and homogeneity of variances. Means were analyzed by the least significant difference test (LSD Fisher) with a $p \leq 0.05$.

2.7. Principal Component Analysis

A principal component analysis (PCA) was conducted using PAST 3.24 software to assess the relationship between the studied parameters. Separate PCA analyses were performed for each factor: microbial inoculation and application of organic amendments.

3. Results

3.1. Strawberry Plant Rooting Percentage (Fragaria sp. var. San Andreas)

The results indicated no interaction between the study factors; however, a significant effect of microbial inoculation on the percentage of strawberry seedling mortality was observed. Plants inoculated with *Trichoderma* sp. and co-inoculated with *Trichoderma* sp. and *Pseudomonas putida* showed lower plant mortality (*p*-value 0.024) compared to non-inoculated plants (Figure 1).

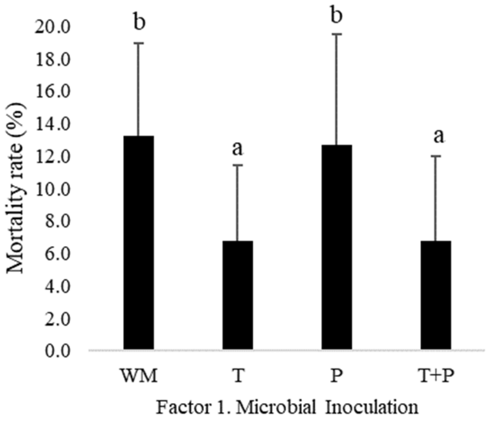
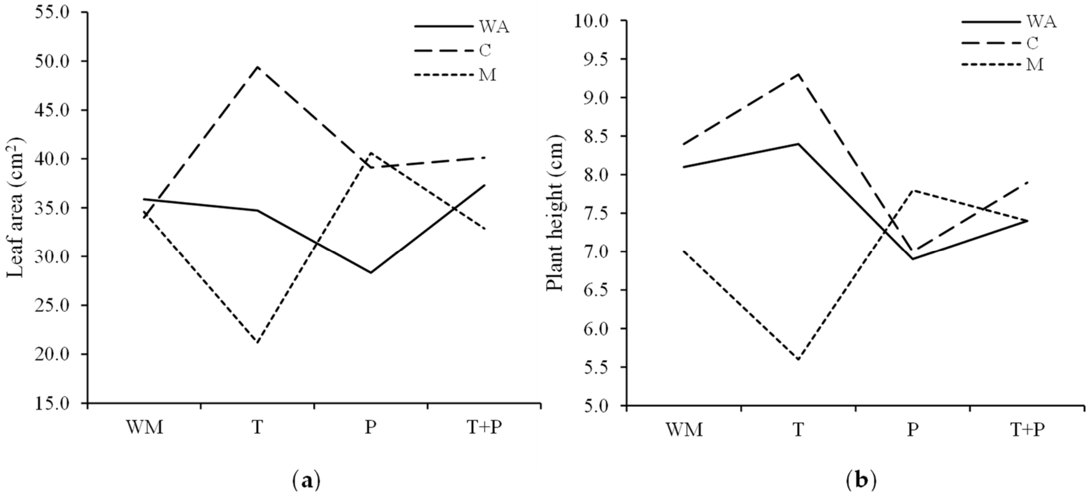


Figure 1. Mortality rates in strawberry seedlings cv. San Andreas at 30 dat according to the microbial inoculation (WM = without microbial inoculation, T = inoculation with *Trichoderma* sp., P = inoculation with *Pseudomonas putida*, T + P = co-inoculation). Means with different letters are statistically different (LSD Fisher, α = 0.05.)

3.2. Number of Leaves, Plant Height, and Leaf Area

A significant interaction of factors was observed in the initial evaluations for leaf area (*p*-value 0.048) and plant height (*p*-value 0.027) (Figure 2). The combined treatment of inoculation with *Trichoderma* sp. plus compost incorporation was the most significant interaction for both variables. It was also observed that, in both parameters, the organic amendments application has a very variable effect when interacting with *Trichoderma* sp. Figure 2 shows that *Trichoderma* sp. in combination with cow manure plus avocado stubble presents very low values; however, when combined with compost, the height and leaf area values increase. This significant interaction was not maintained until the end of the trial.



(**a**) (**b**)

Figure 2. Interaction between microbial inoculation and the organic amendments application on the (**a**) leaf area at 30 dat and (**b**) plant height at 60 dat of strawberry plants cv. San Andreas at. (WM = without microbial inoculation, T = inoculation with *Trichoderma* sp., P = inoculation with *Pseudomonas putida*, T + P = co-inoculation, WA = without organic amendments, C = with compost, M = with manure and leaf litter.)

Regarding the individual effects of treatment on each parameter, it was observed in Table 2 that, for leaf number, microbial inoculation did not differ from the non-inoculated treatment; in fact, the treatment with *Trichoderma* sp. had the lowest value at 90 dat. In this period, it was observed that the compost application increased the number of leaves (*p*-value 0.047), differing from plants without the organic amendment. None of the factors had a significant effect on plant height at 90 dat.

Table 2. Number of leaves, plant height, and leaf area of strawberry seedlings cv. San Andreas at the end of the vegetative stage (90 dat). (WM = without microbial inoculation, T = inoculation with *Trichoderma* sp., P = inoculation with *Pseudomonas putida*, T + P = co-inoculation, WA = without organic amendments, C = with compost, M = with manure and leaf litter).

Treatment	**Number of Leaves per Plant**	**Plant Height (cm)**	**Leaf Area (cm^2)**
Interaction	n.s.	n.s.	n.s.
	Factor 1. Microbial inoculation		
WM	13.06 ± 1.6 ^{a}	11.89 ± 1.2 n.s.	788 ± 128 ^{ab}
T	10.44 ± 2.3 ^{b}	10.73 ± 1.3	630 ± 140 ^{c}
P	13.24 ± 2.2 ^{a}	11.26 ± 1.4	686 ± 133 ^{bc}
T + P	13.45 ± 3.4 ^{a}	12.17 ± 1.2	847 ± 164 ^{a}
	Factor 2. Organic amendment application		
WA	11.53 ± 1.4 ^{b}	11.16 ± 1.2 n.s.	732 ± 158 n.s.
C	14.12 ± 2.7 ^{a}	11.82 ± 1.4	780 ± 161
M	12.00 ± 3.0 ^{ab}	11.55 ± 1.4	701 ± 167

Means with different letters in the same factor in the same column are statistically different (LSD Fisher, α = 0.05); n.s.: no significance.

The co-inoculated treatment (*Trichoderma* sp. + *Pseudomonas putida*) showed plants with greater leaf area compared to the other treatments (Figure 3); this result was significant at 60 (*p*-value 0.005) and 90 dat (*p*-value 0.012).

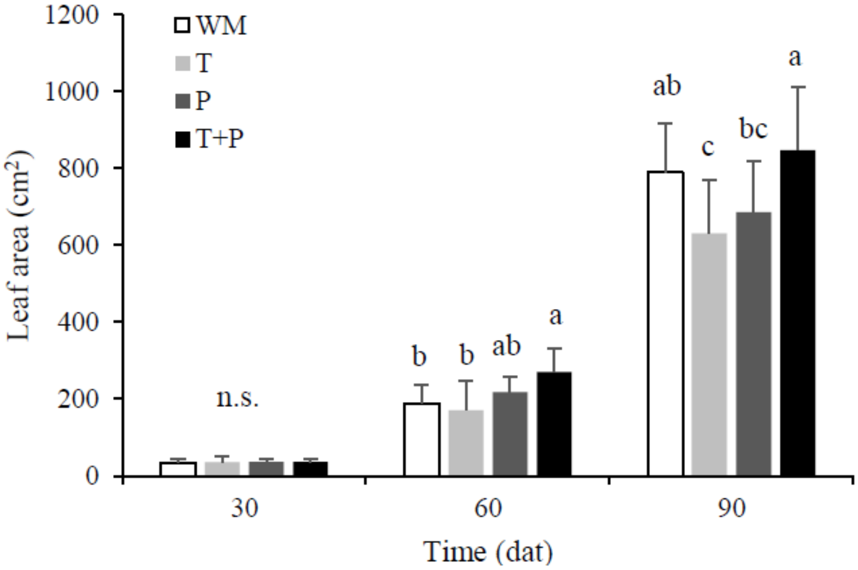


Figure 3. Leaf area according to microbial inoculation from 30 to 90 dat. (WM = without microbial inoculation, T = inoculation with *Trichoderma* sp., P = inoculation with *Pseudomonas putida*, T + P = co-inoculation.). Means with different letters are statistically different (LSD Fisher, α = 0.05.); n.s. no significance.

3.3. Fresh and Dry Biomass

A significant interaction of factors was observed in the aerial (*p*-value 0.040) and root (*p*-value 0.014) dry biomass parameters. The treatment whose seedlings were co-inoculated and had compost application stood out in both parameters (Table S1). This interaction was only significant in the first evaluation (90 dat) and was not maintained towards the end of the trial, so the results were analyzed according to each study factor.

It was observed that microbial inoculation had statistical differences in the fresh (p-value 0.029) and dry biomass (p-value 0.018) of the aerial part at 90 dat (Table 3). In both parameters, co-inoculated seedlings (*Trichoderma* sp. + *Pseudomonas putida*) presented higher aerial biomass, and in the specific case of aerial dry biomass, the co-inoculation treatment was statistically different from the treatment without inoculation. The organic amendments application, specifically compost, was able to increase the aerial dry biomass (p-value 0.008) at 90 dat (Table 3), compared to cow manure and the treatment without amendment (Table 3).

Table 3. Fresh and dry biomass of the aerial part based on microbial inoculation and the organic amendments application. (WM = without microbial inoculation, T = inoculation with *Trichoderma* sp., P = inoculation with *Pseudomonas putida*, T + P = co-inoculation, WA = without organic amendments, C = with compost, M = with manure and leaf litter).

Treatment	Fresh Biomass		Dry Biomass	
	90 dat	**Harvest**	**90 dat**	**Harvest**
	Factor 1. Microbial inoculation			
WM	37.8 ± 9.7 ^{ab}	90.3 ± 13.2 n.s.	7.6 ± 2.3 ^{b}	25.2 ± 5.8 n.s.
T	34.3 ± 6.0 ^{b}	92.2 ± 26.5	8.3 ± 1.4 ^{b}	25.8 ± 5.3
P	33.3 ± 6.2 ^{b}	85.3 ± 26.6	8.1 ± 1.5 ^{b}	23.9 ± 5.2
T + P	42.7 ± 8.5 ^{a}	109.4 ± 26.4	9.7 ± 1.5 ^{a}	29 ± 6.1
	Factor 2. Organic amendment application			
WA	34.5 ± 5.8 n.s.	95.2 ± 16.9 n.s.	7.9 ± 1.4 ^{b}	25.0 ± 4.4 n.s
C	40.7 ± 8.9	93.4 ± 24.1	9.5 ± 1.7 ^{a}	27.2 ± 6.2
M	35.5 ± 8.9	93.3 ± 34.6	7.6 ± 2.0 ^{b}	25.8 ± 6.6

Means with different letters in the same factor in the same column are statistically different (LSD Fisher, α = 0.05); n.s.: no significance.

3.4. Flowering, Fruit, and Yield Analysis

The results showed that the plants with the compost application started flowering in a shorter time compared to the plants with treatment without amendment (p-value 0.007). Also, plants with inoculation with *Pseudomonas putida* (individual and co-inoculated) started fruiting in a shorter time compared to plants with the treatment without inoculation (p-value 0.012) (Figure 4).

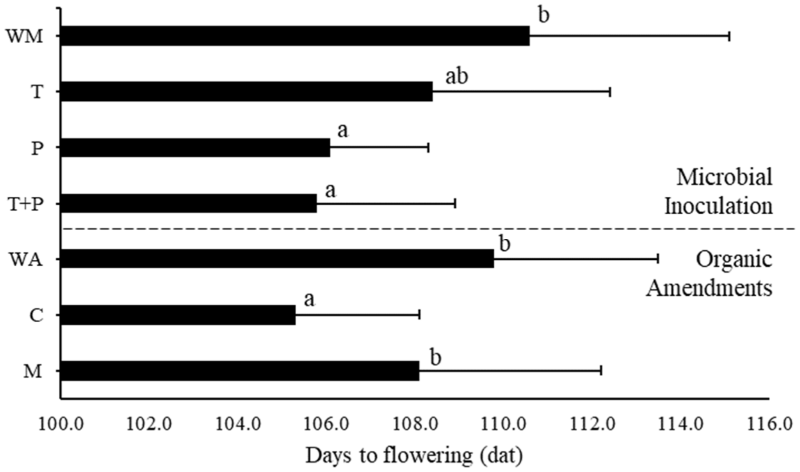


Figure 4. Days to flowering in strawberry seedlings cv. San Andreas based on microbial inoculation and organic amendments application. (WM = without microbial inoculation, T = inoculation with *Trichoderma* sp., P = inoculation with *Pseudomonas putida*, T + P = co-inoculation, WA = without organic amendments, C = with compost, M = with manure and leaf litter.) Means with different letters in the same factor are statistically different (LSD Fisher, α = 0.05).

Regarding flowers and fruits, it was observed that the compost application treatment increased the number of flowers per m^2 (p-value 0.027), while co-inoculation increased the number of fruits per m^2 (p-value 0.037) (Figure 5).

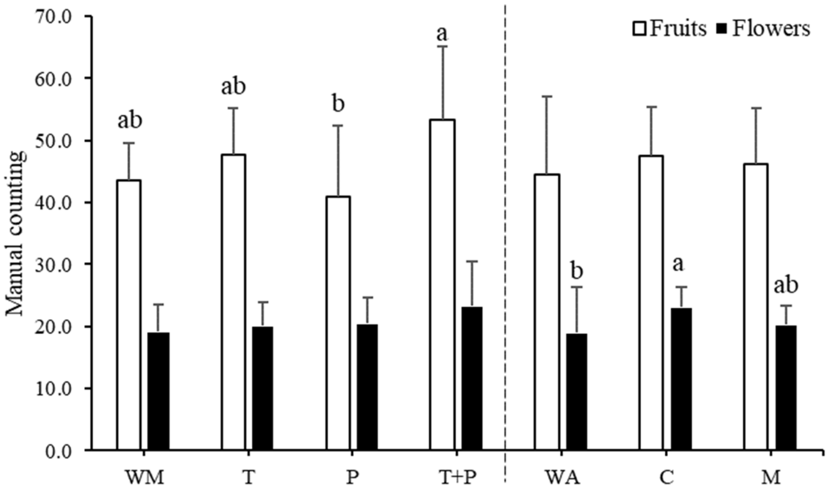


Figure 5. Number of fruits and flowers per square meter in strawberry seedlings cv. San Andreas based on microbial inoculation and the organic amendments application. (WM = without microbial inoculation, T = inoculation with *Trichoderma* sp., P = inoculation with *Pseudomonas putida*, T + P = co-inoculation, WA = without organic amendments, C = with compost, M = with manure and leaf litter.) Means with different letters in the same factor in the same series are statistically different (LSD Fisher, $\alpha = 0.05$).

The fruit's physical–chemical evaluation results are shown in Table 4. Statistical differences were only found in the titratable acidity of the fruits, where the treatment without organic amendment showed slightly more acid fruits (p-value 0.047). Concerning pH, firmness, Brix degrees, and fruit dimensions, it was observed that the study factors maintained the same fruit quality as the control treatments.

Table 4. Physical–chemical characteristics and fruit size based on microbial inoculation and organic amendments application. (WM = without microbial inoculation, T = inoculation with *Trichoderma* sp., P = inoculation with *Pseudomonas putida*, T + P = co-inoculation, WA = without organic amendments, C = with compost, M = with manure and leaf litter).

Treatment	**pH**	**Acidity (g Citric Acid·100 mL^{-1})**	**Sugars (Brix°)**	**Firmness (kg)**	**Size (cm^2)**
Interaction	n.s.	n.s.	n.s.	n.s.	n.s.
Factor 1. Microbial inoculation					
WM	3.37 ± 0.06 n.s.	0.71 ± 0.06 n.s.	13.33 ± 1.80 n.s.	0.14 ± 0.03 n.s.	1471 ± 107 n.s.
T	3.33 ± 0.10	0.76 ± 0.08	12.84 ± 2.22	0.15 ± 0.03	1451 ± 55
P	3.38 ± 0.12	0.73 ± 0.09	13.65 ± 1.83	0.13 ± 0.03	1541 ± 110
T + P	3.40 ± 0.04	0.69 ± 0.04	12.7 ± 2.22	0.16 ± 0.09	1435 ± 179
Factor 2. Organic amendments application					
WA	3.33 ± 0.09 n.s.	0.76 ± 0.07 ^{a}	12.99 ± 1.77 n.s.	0.14 ± 0.03 n.s.	1492 ± 169 n.s.
C	3.39 ± 0.06	0.71 ± 0.06 ^{ab}	13.33 ± 2.12	0.14 ± 0.04	1485 ± 88
M	3.39 ± 0.09	0.69 ± 0.07 ^{b}	13.08 ± 2.17	0.14 ± 0.08	1447 ± 102

Means with different letters in the same factor in the same column are statistically different (LSD Fisher, $\alpha = 0.05$); n.s.: no significance.

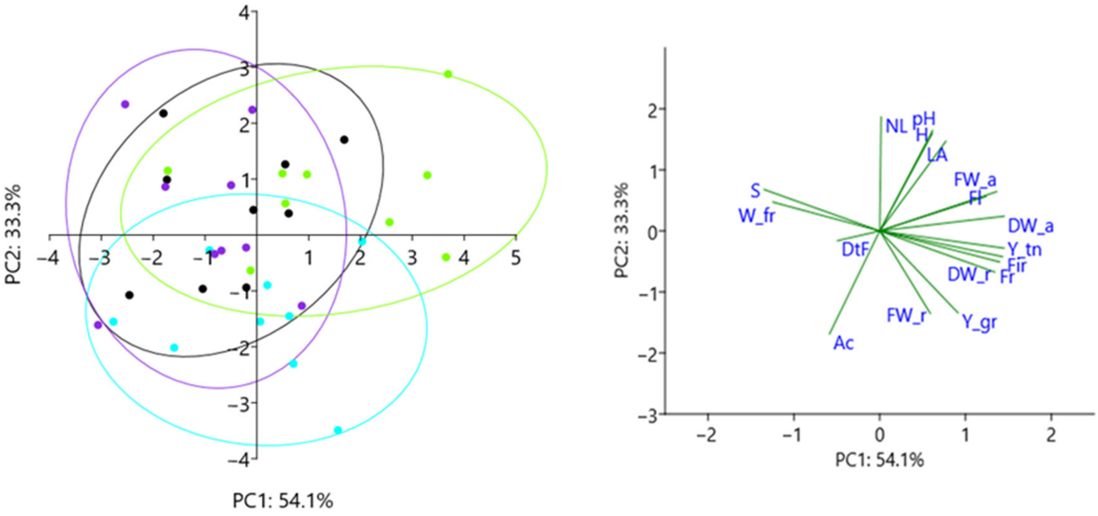
In terms of crop yield, the results showed that the organic amendments application, with either compost or manure, significantly increased the grams of fruit per plant (p-value 0.023). In the microbial inoculation cases, we did not find significant differences (Table 5).

Table 5. Strawberry crop yield cv. San Andreas based on microbial inoculation and the organic amendment application. (WM = without microbial inoculation, T = inoculation with *Trichoderma* sp., P = inoculation with *Pseudomonas putida*, T + P = co-inoculation, WA = without organic amendments, C = with compost, M = with manure and leaf litter).

Treatment	Fruit Weight (g Fruit^{-1})	Yield (g Fruit Plant^{-1})	Yield (t ha^{-1})
Interaction	n.s.	n.s.	n.s.
Factor 1. Microbial inoculation			
WM	21.29 ± 1.15 n.s.	173.6 ± 65.3 n.s.	9.3 ± 1.1 n.s.
T	20.61 ± 1.23	212.9 ± 45.7	9.8 ± 1.5
P	20.96 ± 1.89	183.7 ± 73.7	8.9 ± 3.0
T + P	20.36 ± 1.32	201.2 ± 84.3	10.3 ± 2.0
Factor 2. Organic amendment application			
WA	20.91 ± 1.60 n.s.	152.7 ± 78.0 ^{b}	9.3 ± 2.7 n.s.
C	21.10 ± 1.52	214.4 ± 48.8 ^{a}	9.9 ± 1.6
M	20.41 ± 1.09	211.4 ± 58.4 ^{a}	9.6 ± 1.7

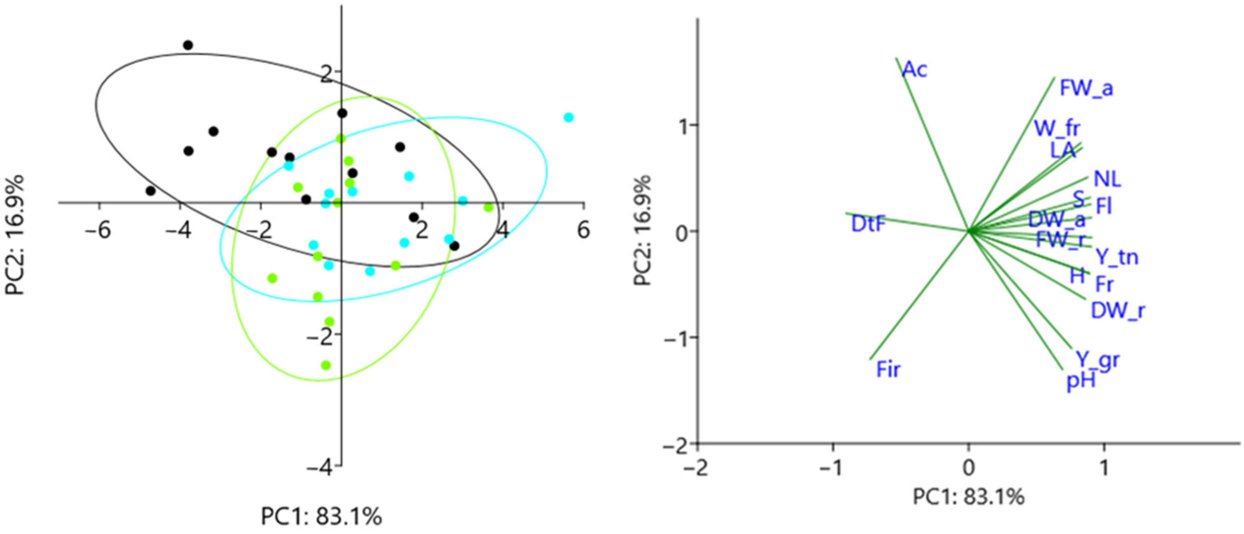
Means with different letters in the same factor in the same column are statistically different (LSD Fisher, α = 0.05); n.s.: no significance.

Figures 6 and 7 show the Principal Component Analysis (PCA) for microbial inoculation and the application of organic amendments, respectively. In both cases, the first two principal components explain over 80% of the total variance. The samples that are on the positive axis of PC1 have a greater influence on most of the variables evaluated (Figures 6b and 7b). On this basis, we observed that co-inoculation (Figure 6a) and the compost application (Figure 7a) are the treatments that had the best results in the variables.



(**a**) (**b**)

Figure 6. Principal Component Analysis (PCA) showing the interrelation of evaluated parameters in strawberries according to microbial inoculation. (**a**) Distribution of inoculation treatments in the principal components (Black = Without inoculation; Aqua = Inoculation with *Trichoderma* sp.; Purple: Inoculation with *Pseudomonas putida*; Green: Co-inoculation). (**b**) Distribution of the variables in the principal components (NL = Number of leaves; H = Height; LA = Leaf area; FW_a = Fresh weight aerial; FW_r = Root fresh weight; DW_a = Dry weight aerial; DW_r = Root dry weight; DtF = Days to fruiting; Fl = Number of flowers; Fr = Number or fruits; W_fr = Fruit weight; Y_gr = Yield expressed in grams of fruit per plant; Y_tn = Yield expressed in tons per hectare; pH = pH; S = Sugars; Ac = Acidity; Fir = Firmness).



(**a**) (**b**)

Figure 7. Principal Component Analysis (PCA) showing the interrelation of evaluated parameters in strawberries according to the application of organic amendments. (**a**) Distribution of inoculation treatments in the principal components (Black: Without organic amendment; Aqua: with compost; Green: with manure and leaf litter). (**b**) Distribution of the variables in the principal components (NL = Number of leaves; H = Height; LA = Leaf area; FW_a = Fresh weight aerial; FW_r = Root fresh weight; DW_a = Dry weight aerial; DW_r = Root dry weight; DtF = Days to fruiting; Fl = Number of flowers; Fr = Number or fruits; W_fr = Fruit weight; Y_gr = Yield expressed in grams of fruit per plant; Y_tn = Yield expressed in tons per hectare; pH = pH; S = Sugars; Ac = Acidity; Fir = Firmness).

4. Discussion

Trichoderma sp. inoculation and the co-inoculation of *Trichoderma* sp. with *Pseudomonas putida* were found to be the treatments with the lowest mortality percentage, less than 7%. Similar results have been reported with the application of effective microorganisms supplemented with bokashi, where strawberries reached less than a 10% mortality rate [22]. In other studies, more than 98% of strawberry seedlings inoculated with *Trichoderma* sp. [23] were able to achieve rooting. However, *Pseudomonas putida* has a higher mortality rate, similar to the uninoculated treatment.

The difference between *Trichoderma* sp. and *Pseudomonas putida* is likely due to the greater efficacy in controlling root diseases and *Trichoderma* sp. causing greater root biostimulation. Root pathogens such as *Phytophthora* sp., *Fusarium* sp., and *Rhizoctonia* sp. are the main limiting factors in strawberry establishment [24]. These cause rotting of the crown and lateral roots. The mechanisms used by *Trichoderma* sp. to control root pathogens are antibiosis, mycoparasitism, induced resistance, and niche exclusion [25]. Previous research shows that glyotoxins, viridin, trichodermin, and furanone are the main metabolites that degrade the cell wall and cause pathogenic hyphae-programmed cell death [26].

Strawberry's high sensitivity to salt stress could cause the low rotting percentage [27] obtained in this research (the maximum value was 93%). Osmotic and oxidative stress caused by a high level of salts inhibits strawberry root development in the early development stages [28]. *Trichoderma* sp. promotes root growth by releasing auxin intermediary metabolites such as indole 3-acetaldehyde acid (IAA) and indole 3-ethanol (IET) [29]. Furthermore, under salt stress conditions, *Trichoderma* sp. produces 6-pentyl-α-pyrone (6-PP), a volatile organic compound that inhibits primary root growth and induces lateral root growth [30]. However, to determine the influence of *Trichoderma* sp. on the mortality rate, more detailed studies should be carried out.

Regarding vegetative growth, it was found that the combination of *Trichoderma* sp. with compost resulted in the highest values for leaf number and plant height during the early growth stages. However, at 90 dat, only the compost explained the highest number of leaves and the co-inoculation of *Trichoderma* sp. and *Pseudomonas putida* explained the highest leaf area. Also, microbial co-inoculation and the compost application increased the aerial dry biomass.

Increased biomass expressed as greater leaf number and plant height is related to a major leaf photosynthetic activity [31]. Sucrolytic activity in *Trichoderma* sp. fungal cells, mediated by intracellular invertase, is known to direct the systemic induction of photosynthesis and sucrose partitioning to the roots [32]. In addition, *Trichoderma* sp. causes plant gene expression reprogramming to induce the rubisco activity and the oxygen-generating complex of the photosystem II [33]. The leaf area increase is likely due to an increased requirement for photosynthetic activity. In addition, synergistic action between *T. harzianum* and *P. fluorescens* in increasing nitrogen and phosphorus uptake has been reported, resulting in higher aerial biomass [34]. Both *Pseudomonas* sp. and *Trichoderma* sp. can exert beneficial and biocontrol effects on plants; however, studies show that combinations of the two are more effective than each individually [35,36]. This situation was observed in our results, as co-inoculated seedlings showed greater leaf area and aerial biomass, statistically different from those in which only one microorganism was inoculated. In addition, compost use as an organic amendment or substrate for *Trichoderma* sp. development resulted in greater plant growth stimulation. This positive effect of compost agrees with other studies, where the incorporation of organic amendments in strawberry cultivation increases the number of leaves [37].

The positive effect of co-inoculation could be associated with synergism between bioactive molecules or volatile organic compounds of both strains [38]. Biomass increase due to the integrated effect of different rhizospheric strains has been reported in strawberry cultivation [39]. Also, this response could be attributed to chlorophyll content increase, an indirect indicator of leaf photosynthetic activity [40]. This study revealed the positive effect of the interaction between compost and co-inoculation on biomass increase. There is scientific evidence justifying the increased nitrogen mineralization and phosphatase activity in the soil due to the inoculation effect of *Trichoderma* sp. supplemented with compost [41]. Also, studies indicate that the application of compost improves microbial activity and thus generates the production of growth regulators by microorganisms, which is reflected in an increase in biomass in the plant. [42]. This is supported by other studies with strawberry seedlings grown under greenhouse conditions, where it is shown that compost helps *Trichoderma* sp. populations to remain stable in the soil [43].

Inoculation with *Pseudomonas putida* (individual or co-inoculation with *Trichoderma* sp.) influenced the days to flowering, reducing the time by an average of 4 days. These results have been rarely reported in fruit crops; however, it has been found in the literature that *Pseudomonas* sp. influences flowering. In a study on the chrysanthemum, *Pseudomonas* sp. accelerated flowering by an average of 14 days compared to the control [44]. Another study on the chrysanthemum showed that two strains of *Pseudomonas putida*, characterized as indole acetic acid (IAA) producers, improved flowering [45]. So, it is likely that this influence is related to the improvement in nutrient absorption and the transport of growth-promoting substances, resulting in an early transformation of plant parts from the vegetative to the reproductive phase.

On the other hand, organic amendments not only provide nutrients to the plant but also contribute organic matter, thereby increasing soil fertility [46]. The addition of compost is known to stimulate microbial growth due to the increased amount of organic carbon it provides [47]. It can also have a direct effect on the plant due to the microorganisms present in it. Manure improves soil's chemical and physical properties such as soil water, bulk density, erosion resistance, and nutrient status, among others, which can favor crop growth and productivity [48]. The benefits of the amendments application were reflected in the yield increase (g fruit plant^{-1}) observed in our results, which correlates with what is found in the literature. Some studies conclude that the addition of vermicompost improved growth and yield levels in strawberries [49,50]. Also, significant differences in strawberry yield and fruit quality were found with the addition of chicken manure [51].

5. Conclusions

Pseudomonas putida inoculation accelerated the seedling's flowering process and co-inoculation (*Trichoderma* sp. + *Pseudomonas putida*) increased the leaf area, fresh biomass, and dry biomass, both aerial and root, and the number of fruits per square meter. Concerning the organic amendments, only the compost treatment stood out, with higher values found in the number of leaves, the aerial dry biomass, the days to flowering, the number of flowers per square meter, and the fruit yield per plant. Under the conditions in which the experiments were carried out, these results show that compost application at 15 $t \cdot ha^{-1}$ and co-inoculation of *Trichoderma* sp. and *Pseudomonas putida* have potential as organic technologies in *Fragaria* sp. cultivation; however, it is important to repeat these treatments in other seasons and regions to validate the effect observed in our study.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/applmicrobiol4030075/s1, Table S1: Aerial and root dry biomass at 90 dat based on the interactions of the two study factors.

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