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Mycorrhizal Biotechnology Reduce Phosphorus in the Nutrient Solution of Strawberry Soilless Cultivation Systems

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Abstract: Among the bio-tools that contribute to making the strawberry production system more sustainable are arbuscular mycorrhizal fungi (AMF), which can be used as biofertilizers. Thus, five doses of phosphorus (P) applied to plants with AMF and a control (100% P, no AMF) were analyzed in order to differentiate the horticultural potential of the 'Camarosa' cultivar. We used an on-farm inoculant made up of six fungal species. The univariate and multivariate analyses showed that the addition of AMF to the growing substrate made it possible to reduce the P supply in the nutrient solution by 75% without compromising the fruit yield. In addition, this combination improved the phytochemical quality of strawberries, the plant's root system morphology, and the accumulation of nutrients in plant organs (roots, crowns, aerial part and fruits). We conclude that the use of a multi-species on-farm inoculant based on AMF associated with a reduction in the P supply in the nutrient solution modifies the horticultural potential of the 'Camarosa' cultivar. We confirmed the action of a native AMF community as a biofertilizer.

Keywords: *Fragaria* × *ananassa* Duch.; yield; quality; root system; nutritional composition

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1. Introduction

As an initiative to improve fruit yields in the Brazilian subtropics, strawberry (*Fragaria* × *ananassa* Duch.) cultivation is migrating to the soilless, substrate system [1]. In this system, between 84 and 90% of the total phosphorus (P) supplied to the plants via phosphate fertilizers added to the nutrient solution is lost due to the rapid reaction with the growth medium's components, which fix the nutrient and make it unavailable to the plants [2]. In addition to being part of all cells' genetic material, P is involved in energy transfer, photosynthesis, sugar transformation, and nutrient movement within the plant [3]. P-deficient plants develop small, unstretched new leaves, dark green in color; old leaves with a gradual purplish color from the outside to the inside; and undifferentiated flower buds [4].

New nutritional management alternatives should be proposed to minimize these drawbacks, such as inoculation with arbuscular mycorrhizal fungi (AMF). The symbiosis between these microorganisms and the strawberry plant increases the P acquisition efficiency, which can help to reduce phosphate fertilization and benefit fruit production [5].

Thus, AMF use in strawberry cultivation can function as an economically and environmentally viable fertilization technique [6].

AMF are able to increase the P acquisition by plants, especially in conditions of low availability of the nutrient in the growth medium. P acquisition by AMF is mediated by extraradical hyphae and fungal phosphate transporters [7]. Fungal homologous genes of Pi:H^+ symporter are expressed in extraradical mycelium (ERM). After being absorbed by the ERM, inorganic phosphorus (Pi) is converted into polyphosphate chains (polyP) within the vacuole [8]. PolyP is translocated into the intraradical mycelium (IRM) by cytoplasmic flow and/or along a mobile tubular vacuolar network [9]. After the hydrolysis that occurs in the arbuscule, Pi is exported from the AMF to the periarbuscular space. The breakdown of PolyP in IRM possibly involves acid and alkaline phosphatases [10]. Pi import across the periarbuscular membrane into the root cell is mediated by Pi transporters: mycorrhizal-induced Pht1, subfamily III (e.g., LjPT3) [11], and mycorrhizal-specific Pht1, subfamily I (e.g., LePT4) [12]. This dynamic and complex interface between AMF and plants can contribute to sustainable horticulture.

As excessive P doses can reduce mycorrhizal association [13], we need to adjust the amounts of this nutrient added to favor symbiosis with plants grown in substrates. When P availability increases, plants do not produce the molecular signals (gene activation, enzymatic activity, and protein production) necessary for the establishment of symbiosis [14]. Thus, we must modify the management traditionally adopted in the substrate cultivation system, such as the large-scale use of phosphate fertilizers. This could enhance the beneficial effects of mycorrhizal biotechnology on strawberry yield and quality. In this way, we can establish a balance between economic/productive efficiency and the preservation of natural resources.

Our hypothesis is that the decrease in P supply could no longer be unfavorable for strawberry growth, development, and quality when AMF is provided. Therefore, here, we investigated whether the production, fruit quality, root system morphology, and nutritional composition of organs differ among strawberry plants, 'Camarosa' cultivar, in the absence (−) and presence (+) of AMF with a reduction in the P supply in the nutrient solution. In order to understand the relationship between the treatments studied and the attributes evaluated, we explored the data using multivariate analysis. The research may help provide more clues for the improvement of nutrient management strategies in strawberry production and produce more environmentally friendly strawberries by reducing the use of phosphate chemical fertilizers.

2. Materials and Methods

2.1. Plant Material

The research was carried out at the University of Passo Fundo (28°15′46′′ S; 52°24′24′′ W, altitude 687 m), Rio Grande do Sul (RS), Brazil, from August (winter) to December (summer), in a greenhouse (430 m^2) with a semicircular roof covered with low-density polyethylene film (thickness: 150 μm and anti-ultraviolet additive).

In August, strawberry plants of the 'Camarosa' cultivar from the Llahuén nursery in Chile (33°50′15.41′′ S; 70°40′03.06′′ W, altitude 115 m) were transplanted into containers with Horta 2® substrate (MecPlant, Telêmaco Borba, Brazil) in order to produce stolons. 'Camarosa' is classified as a short-day flowering plant. The plants were kept in a greenhouse on 1.20 m high benches. Fertigation was provided to the plants every two weeks [15].

After seven months, in March (fall), we removed stolons from the mother plants with crown diameters between 2 and 5 mm, with two expanded and intact leaves, and uninjured petioles and root primordia between 0.5 and 1.0 cm long [16]. These stolons were transferred to a polystyrene tray filled with sterilized Horta 2® substrate (MecPlant, Telêmaco Borba, Brazil) (120 °C, 20 min) to obtain the strawberry daughter plants that constituted the plant material for the research. The stages of obtaining the daughter plants are shown in Figure 1.

Figure 1. Obtaining strawberry daughter plants used as plant material for the study.

During acclimatization, the daughter plants were irrigated using a sprinkler system. The supply of nutrient solution began 15 days after rooting. The macronutrients were supplied in concentrations (mg.plant^{-1}) of: 162 of N, 144 of K, 96 of Ca, 29 of Mg, and 35 of S. The pH of the nutrient solution was kept at 5.5 and the electrical conductivity (EC) at 1.5 dS.m^{-1}. The fertilizers used were potassium nitrate (KNO_3), calcium nitrate [$Ca(NO_3)_2$], and magnesium sulfate ($MgSO_4$).

2.2. Experimental Design

The treatments consisted of five doses of P (0, 34, 69, 137, and 274 mg.plant^{-1}) applied by means of fertigation throughout the phenological stages of plants inoculated with an AMF community and a control treatment (plants not inoculated with AMF and fertigated with the 274 mg.plant^{-1} dose of P). The experimental design used was entirely randomized, with 20 replications. Each plot consisted of four plants (80 plants per treatment; n = 480 plants).

The amount of P (and other nutrients) applied in the control treatment corresponded to the assumed extraction dose for this nutrient during the crop cycle (Table 1). These quantities were based on average extraction values indicated in the literature [17,18].

Table 1. Average extraction of N, P, K, Ca, and Mg in strawberry according to their phenological stages.

Phenological Stages [1]	N	P	K	Ca	Mg
	mg.plant^{-1}				
Vegetative	162	33	144	96	29
Beginning of flowering	556	84	421	225	67
Beginning of fruiting	721	134	700	331	110
Full fruition	1121	205	1230	546	192
End of fruiting	1422	274	1755	604	262

Source: Souza et al. [17] and Tagliavini et al. [18]. [1] Phenological scale proposed by Meier et al. [19] and with the codification of Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie (BBCH).

To adjust the supply of nutrients throughout the stages, the fertigation times corresponding to the volume of nutrient solution were calculated. The P doses were established based on the proportions of 0% (0 mg.plant^{-1}), 12.5% (34 mg.plant^{-1}), 25% (69 mg.plant^{-1}), 50% (137 mg.plant^{-1}), and 100% (274 mg.plant^{-1}) of the amount of this nutrient applied in the control treatment (Table 2).

Table 2. Absence (−) and presence (+) of AMF with reduced P supply in the nutrient solution.

Treatments	P [1] (mg.plant^{-1})	AMF
0% P + AMF	0	Yes (+)
12.5% P + AMF	34	Yes (+)
25% P + AMF	69	Yes (+)
50% P + AMF	137	Yes (+)
100% P + AMF	274	Yes (+)
100% P − AMF	274	No (−)

[1] Phosphorus was supplied to the strawberry plants in the form of KH_2PO_4.

The mycorrhizal community used in the experiment was obtained through the on-farm production of an inoculant, chosen on the basis of a survey of AMF in soil cultivated with strawberry ('Camarosa' cultivar [20]) composed of six species [21]: *Acaulospora mellea* (Spain and N.C. Schenck); *Acaulospora morrowiae* (Spain and N.C. Schenck); *Cetraspora pellucida* (T.H. Nicolson and N.C. Schenck) Oehl, F.A. Souza, and Sieverd.; *Claroideoglomus etunicatum* (W.N. Becker and Gerd.) C. Walker and A. Schüßler, *Glomus* sp.; and *Septoglomus viscosum* (T.H. Nicolson) C. Walker et al.

2.3. Obtaining of on-Farm Inoculant Based on AMF

On-farm inoculant was obtained using the trap culture technique [22], with sorghum (*Sorghum bicolor* (L.) Moench) as the host plant and sterilized sand (120 °C, 20 min) as a substrate.

After five months of sorghum multiplication, the inoculant based on AMF was obtained and contained spores, root pieces (from the trap culture technique), and sterile sand (from the substrate of trap culture technique). Manually, we applied the inoculants in the planting bed of the strawberry daughter plants.

2.4. Procedures

The daughter plants were transplanted in May (autumn) into containers (18 L) filled with sterilized sand (120 °C, 20 min) in the open nutrient solution drainage system. For the treatments inoculated with AMF, 10 g of on-farm inoculant was added to the daughter plants' planting bed at the time of transplanting. The daughter plants were spaced at 0.20 m × 0.25 m and arranged in two rows. The plants were kept in a greenhouse (430 m^2) on benches 1.20 m high.

The nutrient solutions corresponding to each treatment (Table 3) were applied weekly through drip lines with a flow rate of 8 L.h^{-1}. All plants received the following micronutrients (mg.plant^{-1}): B (1.8), Cu (0.18), Fe (36), Mn (12), Mo (0.18), and Zn (0.6). Regardless of the changes in the nutrient solution according to the strawberry plants' phenological stages (Table 3) [19], the potential of hydrogen (pH) was maintained at 6.0 throughout the plants' cultivation. To maintain this pH value, we used hydrochloric acid (HCl, 98%) (for pH > 6.0) and sodium hydroxide (NaOH, 10%) (for pH < 6.0). A mini weather station installed inside the greenhouse was used to monitor the average air temperature and photosynthetically active radiation (PAR) during the experiment (Figure 2).

2.5. Mycorrhizal Colonization

To verify AMF's infective capacity, root portions of plants were prepared [23], and their percentage of mycorrhizal colonization (MC, %) was determined using Equation (1) [24]:

$$\text{MC } (\%) = \frac{\text{total number of fragments with mycorrhizal roots}}{\text{total number of fragments}} \times 100 \quad (1)$$

Table 3. Nutrient solutions used throughout the phenological stages of strawberries, 'Camarosa' cultivar.

Phenological Stages [1]	Doses of P (mg.plant^{-1})	Nutritional Solution Formulations [2]				
		$Ca(NO_3)_2$	KH_2PO_4	KNO_3	$MgSO_4$	NH_2CONH_2
		mg.L^{-1}				
Beginning of flowering	0	507	0	378	276	83
	34	507	19	363	278	87
	69	507	38	348	279	91
	137	507	76	318	283	99
	274	507	152	258	289	115
	0	676	0	730	344	449
	34	676	29	707	346	455
	69	676	57	685	349	461
	137	676	114	640	354	473
	274	676	229	550	364	497
Full fruition	0	557	0	733	403	0
	34	557	29	711	405	6
	69	557	57	688	408	12
	137	557	114	643	413	24
	274	557	228	553	423	48
	0	1134	0	1394	751	127
	34	1134	40	1363	755	135
	69	1134	80	1331	758	144
	137	1134	160	1268	765	161
	274	1134	321	1141	779	194
	0	303	0	1382	629	196
	34	303	40	1351	633	204
	69	303	79	1320	636	213
	137	303	159	1257	643	229
	274	303	317	1132	657	263

[1] Phenological scale proposed by Meier et al. [19], and with the codification of Biologische Bundesanstalt, Bundessortenamt, und CHemische Industrie (BBCH). [2] $Ca(NO_3)_2$: calcium nitrate; KH_2PO_4: potassium monobasic phosphate; KNO_3: potassium nitrate; $MgSO_4$: magnesium sulfate; NH_2CONH_2: urea.

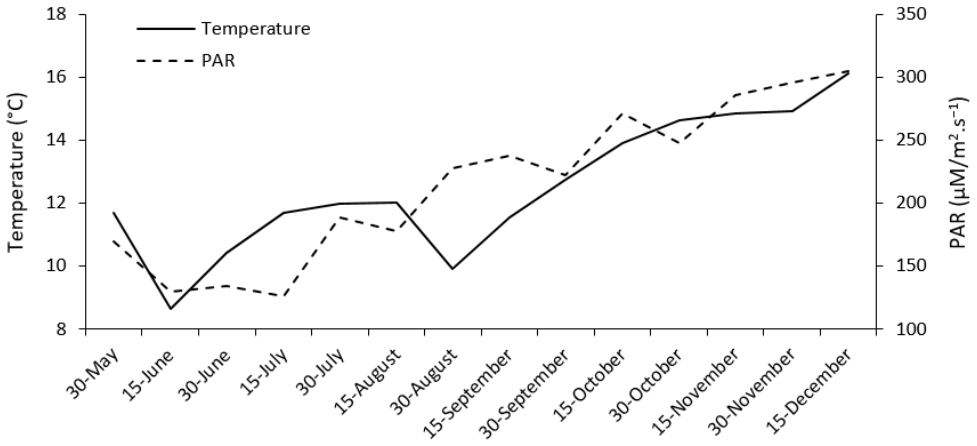

Figure 2. Temperature and PAR averages recorded in the greenhouse during the experimental period.

2.6. Strawberry Production

Between August and December, with approximately eight monthly harvests, we assessed the total number of fruits per plant (TNF, number per plant) and the total fruit production per plant (TP, grams per plant) based on commercial ripeness ($\geq$85% reddish visual color). The fruit was weighed on an electronic digital scale. We also determined the average fresh fruit mass (AFFM, grams) using the ratio between TP and TNF.

2.7. Fruit Quality

The chemical quality analysis was carried out in October (spring). The total soluble solids content (TSS, %) and total titratable acidity (TTA, % of citric acid) were evaluated using 15 fruits from each treatment for each repetition [25]. To assess fruit taste, the TSS/TTA ratio was determined [26].

As for determining the total phytochemical content, also in October, 100 g of fruit from each repetition was used for analysis of total anthocyanins (TAN), total flavonoids (TFL), and total polyphenols (TPO). The fruit was analyzed in fresh mass, which is how it is used for consumption.

TAN was carried out using the pH differential [27]. Readings were taken at 510 nm and 700 nm by spectrophotometry (PerkinElmer Lambda 20, Perkin Elmer®). The TAN content was determined using Equation (2):

$$A = (A_{\lambda vis - max(510)} - A_{700})\ pH\ 1.0 - (A_{\lambda vis - max(510)} - A_{700})\ pH\ 4.5 \quad (2)$$

Equation (3) was used to calculate the monomeric anthocyanin concentration:

$$TAN = \frac{A \times MW \times DF \times 100}{\varepsilon \times 1} \quad (3)$$

where MW = molecular weight of pelargonidin-3-*O*-glucoside (433.20); DF = dilution factor; and ε = molar absorptivity coefficient (25.660). The results were expressed in milligrams of pelargonidin-3-*O*-glucoside equivalent per 100 g of fresh fruit (mg PE/100 g FF^{-1}).

TFL was carried out according to Popova et al. [28], and the results are expressed in milligrams of rutin equivalent per 100 g of fresh fruit (mg RE/100 g FF^{-1}).

TPO was determined using the Folin–Ciocalteu reagent [29]. Absorbance readings were taken at 760 nm. The results were calculated using a standard curve with gallic acid and expressed in milligrams of gallic acid equivalent per 100 g of fresh fruit (mg GAE/100 g FF^{-1}).

2.8. Root System Morphology

At the end of the experiment, in December, the roots were collected and washed in water to remove substrate fragments. The roots were scanned and the images obtained were analyzed using WinRHIZO® software version 2013 (Regent Instruments Inc., Québec City, QC, Canada). The attributes evaluated were the total root length (TRL, cm), superficial area (SA, cm^2), root volume (RV, cm^3), and root diameter (RD, mm). The roots were grouped using the software into different diameter classes in relation to their total length [30]: very thin roots (VTR, Ø < 0.5 mm), fine roots (FR, Ø from 0.5 to 2 mm), and thick roots (TR, Ø > 2 mm).

2.9. Nutritional Composition of Plant Organs

In December, we assessed the macronutrient content (N, P, K, Ca, and Mg), expressed in milligrams per gram of dry mass (mg/g DM^{-1}), in roots, crowns, aerial part, and fruits, following the methodology of Tedesco et al. [31]. The plants were collected, washed, and separated into parts according to their organs (roots, crowns, aerial part, and fruits). These organs were dried in a forced-air oven at 65 °C until reaching a constant weight and ground in a Willey-type knife mill. The samples were then digested with hydrogen peroxide (H_2O_2), sulfuric acid (H_2SO_4), and a digestion mixture (sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$), and selenium (Se)). N contents were determined using the Kjeldahl method, P contents were obtained via spectrophotometry, K contents were determined via flame photometry, and Ca and Mg contents were obtained via atomic absorption spectrophotometry.

2.10. Data Analysis

The data were subjected to analysis of variance (ANOVA), and the means of the treatments were compared using the Duncan test at a 5% probability of error with the Costat® program [32].

We also carried out a multivariate analysis using principal components (PCA), performed after standardizing the attributes, in which each one had a mean of 0 and a variance of 1. We disregarded eigenvalues below 1 from the analysis, as they did not provide relevant information [33]. We ran the PCA with the '*factoextra*' package in RStudio [34].

To illustrate the relationship among the six treatments in terms of yield, fruit quality, root system morphology, and nutritional composition of organs in strawberry plants, we used hierarchical cluster analysis (using the single linkage method between groups—nearest neighbor) calculated by the Euclidean distance. The results are presented in a dendrogram elaborated with the '*stats*' package [34]. To validate the cluster analysis, we obtained the cophenetic correlation coefficient (CCC), which is a measure of the correlation among the elements of the original dissimilarity matrix and their respective elements of the matrix produced by the dendrogram, known as the cophenetic matrix [35].

3. Results

3.1. Mycorrhizal Colonization

Reducing P supply in the nutrient solution increased mycorrhizal colonization, which confirms and highlights the effect of P fertilizer on these microorganisms. There was no AMF contamination in the control treatment (Figure 3).

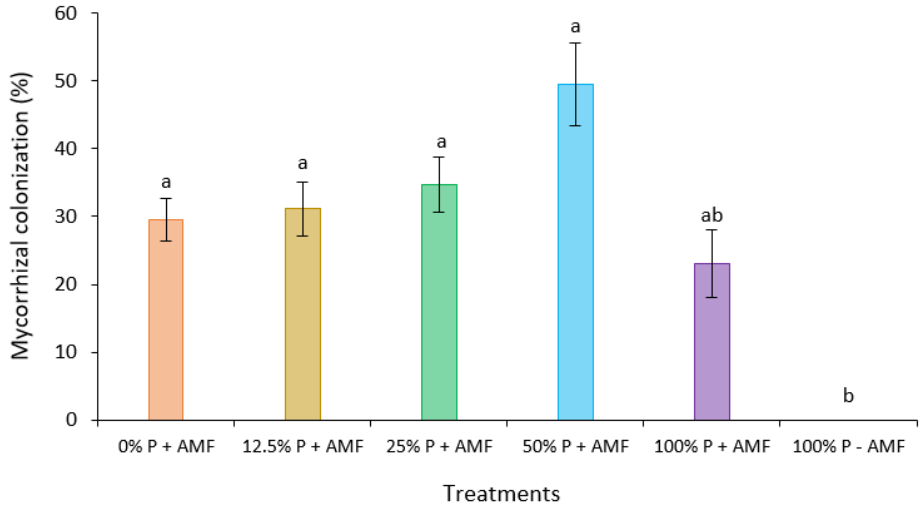

Figure 3. Mycorrhizal colonization of strawberry plants cultivated in the absence (−) and presence (+) of AMF with reduced supply of P in the nutrient solution. Data are presented as mean ± standard deviation. Means followed by the same letter in the column did not differ between each other according to Duncan's test ($p \leq 0.05$).

The structures observed on the roots of the strawberry plants, regardless of the treatment analyzed, were hyphae and arbuscules (Figure 4).

3.2. Strawberry Production

We observed a significant treatment effect only for the TP and AFFM attributes. The use of a multi-species on-farm inoculant made it possible to reduce P supply in the nutrient solution by up to 75% (25% P + AMF) without compromising strawberry yields (Table 4).

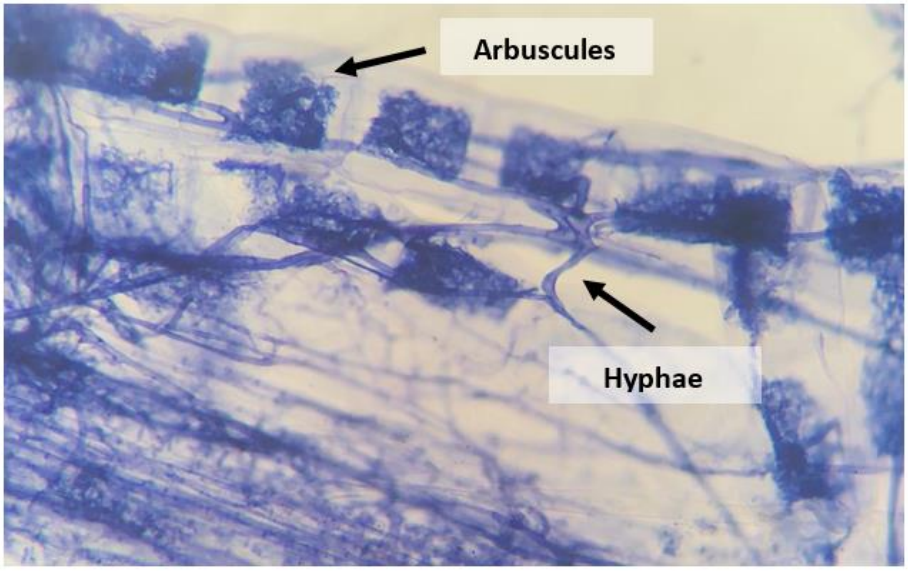

Figure 4. Hyphae and arbuscules visualized in strawberry roots. Optical microscope observation, with magnification of 400×.

Table 4. Fruit production of plants cultivated in the absence (−) and presence (+) of AMF with reduced supply of P in the nutrient solution.

Treatments	TNF [1] (Number per Plant)	TP (Grams per Plant)	AFFM (Grams)
0% P + AMF	11.25 ± 1.95	149.74 ± 52.88 c	14.66 ± 2.48 b
12.5% P + AMF	13.87 ± 3.15	223.79 ± 49.14 b	15.07 ± 2.98 b
25% P + AMF	18.25 ± 2.85	310.13 ± 52.18 a	16.99 ± 3.65 a
50% P + AMF	16.75 ± 2.91	282.58 ± 57.91 a	16.84 ± 5.11 a
100% P + AMF	18.43 ± 2.87	317.46 ± 46.12 a	17.18 ± 2.92 a
100% P − AMF	19.14 ± 2.06	326.57 ± 48.74 a	15.11 ± 1.43 b
Significance	NS	**	**
Mean	16.52	268.38	16.14
CV [2] (%)	8.19	11.82	6.20

Data are presented as mean ± standard deviation. Means followed by the same letter in the column did not differ between each other according to Duncan's test ($p \leq 0.05$). **: significant at the 1% probability level ($p < 0.01$). NS: treatment effect not significant ($p > 0.05$). [1] TNF: total number of fruits; TP: total production; AFFM: average fresh fruit mass. [2] CV: coefficient of variation.

Plants grown with AMF and 75% P reduction (25% P + AMF) had TP (grams per plant) only 5% lower compared to those treated with 100% P and without mycorrhizae (100% P − AMF) (Table 4). However, the 25% P + AMF treatment generated fruits with AFFM (grams) higher by 11% compared to the 100% P − AMF treatment (Table 4).

3.3. Fruit Quality

The treatments tested did not influence the attributes of TSS, TTA, TSS/TTA, or TAN. However, all the plants grown with AMF produced fruit with higher TFL and TPO, with the most notable reductions being 87.5% (12.5% P + AMF) and 75% P in the nutrient solution (Table 5).

Plants treated with 12.5% P + AMF and 25% P + AMF produced fruits with an average of 55% and 28% more TFL (mg RE/100 g FF^{-1}) and TPO (mg GAE/100 g FF^{-1}), respectively, in relation to plants grown with 100% P − AMF (Table 5).

Table 5. Fruit quality of plants cultivated in the absence (−) and presence (+) of AMF with reduced supply of P in the nutrient solution.

Treatments	TFL [1] (mg RE/100 g FF^{-1})	TPO (mg GAE/100 g FF^{-1})	TAN (mg PE/100 g FF^{-1})
0% P + AMF	26.52 ± 15.14 ab	713.28 ± 99.45 ab	5.06 ± 0.21
12.5% P + AMF	34.33 ± 19.43 a	810.45 ± 85.92 a	5.55 ± 0.32
25% P + AMF	27.95 ± 18.63 a	780.71 ± 99.32 a	5.86 ± 0.19
50% P + AMF	16.88 ± 19.34 b	659.07 ± 84.12 b	6.34 ± 0.95
100% P + AMF	18.94 ± 15.28 b	636.80 ± 77.81 b	5.78 ± 0.57
100% P − AMF	14.16 ± 19.87 c	575.87 ± 92.23 c	3.70 ± 0.34
Significance	**	**	NS
Mean	23.30	696.03	5.38
CV [2] (%)	14.21	14.23	38.23
Treatments	**TSS (%)**	**TTA (%)**	**TSS/TTA**
0% P + AMF	7.59 ± 0.23	0.54 ± 0.02	14.17 ± 1.49
12.5% P + AMF	7.16 ± 0.12	0.64 ± 0.01	11.83 ± 2.01
25% P + AMF	5.96 ± 0.17	0.39 ± 0.01	15.17 ± 1.56
50% P + AMF	7.12 ± 0.22	0.36 ± 0.02	20.11 ± 1.63
100% P + AMF	6.93 ± 0.14	0.38 ± 0.01	18.53 ± 1.95
100% P − AMF	6.30 ± 0.18	0.41 ± 0.03	15.32 ± 2.08
Significance	NS	NS	NS
Mean	6.84	0.45	15.85
CV (%)	9.56	22.20	15.28

Data are presented as mean ± standard deviation. Means followed by the same letter in the column did not differ between each other according to Duncan's test ($p \leq 0.05$). **: significant at the 1% probability level ($p < 0.01$). NS: treatment effect not significant ($p > 0.05$). [1] TFL: total flavonoids; TPO: total polyphenols; TAN: total anthocyanins; TSS: total soluble solids; TTA: total titratable acidity; TSS/TTA: flavor. [2] CV: coefficient of variation.

3.4. Root System Morphology

AMF, under different P supply levels in the nutrient solution treatments, significantly affected the thin and fine roots. All the plants grown with AMF developed more robust root systems, with greater amounts of VTR and FR, with the most notable reductions being 100% (0% P + AMF), 87.5%, and 75% of P in the nutrient solution (Table 6).

Table 6. Root system morphology of plants cultivated in the absence (−) and presence (+) of AMF with reduced supply of P in the nutrient solution.

Treatments	TRL [1] (cm)	SA (cm^2)	RV (cm^3)	VTR (cm)	FR (cm)	TR (cm)
0% P + AMF	2626.35 ± 136.28	711.25 ± 90.12	15.37 ± 1.07	1931.03 ± 135.17 a	499.33 ± 83.81 a	195.73 ± 41.97
12.5% P + AMF	2892.51 ± 140.93	825.59 ± 88.48	19.14 ± 1.32	2037.55 ± 129.27 a	621.19 ± 81.43 a	233.41 ± 52.82
25% P + AMF	2130.14 ± 138.65	612.66 ± 91.21	14.13 ± 1.75	1565.27 ± 148.27 a	415.43 ± 87.59 a	149.01 ± 49.81
50% P + AMF	1023.73 ± 121.12	483.70 ± 87.06	18.87 ± 2.02	679.72 ± 89.21 b	217.28 ± 78.91 b	126.54 ± 41.18
100% P + AMF	861.98 ± 119.84	416.26 ± 83.93	21.38 ± 2.77	506.01 ± 95.45 b	197.37 ± 97.38 b	158.32 ± 50.15
100% P − AMF	743.02 ± 112.76	397.42 ± 95.73	17.18 ± 1.98	423.57 ± 89.04 c	103.44 ± 72.52 c	125.81 ± 62.08
Significance	NS	NS	NS	**	**	NS
Mean	1712.95	574.48	17.68	1197.19	350.67	164.80
CV [2] (%)	20.00	21.62	26.89	19.36	15.25	22.23

Data are presented as mean ± standard deviation. Means followed by the same letter in the column did not differ between each other according to Duncan's test ($p \leq 0.05$). **: significant at the 1% probability level ($p < 0.01$). NS: treatment effect not significant ($p > 0.05$). [1] TRL: total root length; SA: superficial area; RV: root volume; VTR: very thin roots; FR: fine roots; TR: thick roots. [2] CV: coefficient of variation.

Plants treated with 0% P + AMF, 12.5% P + AMF, and 25% P + AMF showed root systems with an average of 77% and 80% more VTR (cm) and FR (cm), respectively, in relation to plants grown with 100% P − AMF (Table 6).

3.5. Nutritional Composition of Plant Organs

The use of the AMF community made it possible to reduce the P supply in the nutrient solution by up to 75% without compromising the nutritional composition of the plant organs, as this association provided accumulations of N, P, K, Ca, and Mg in the crown; P and Ca in the aerial part; and P, K, and Mg in the fruits that were the same in relation to the highest doses of P in the presence (50% P and 100% P) or absence (100% P) of AMF (Table 7).

Table 7. Nutritional composition of organs of plants cultivated in the absence (−) and presence (+) of AMF with reduced supply of P in the nutrient solution.

Treatments	CNC [1] (mg/g DM^{-1})	CPC (mg/g DM^{-1})	APPC (mg/g DM^{-1})	FPC (mg/g DM^{-1})	CKC (mg/g DM^{-1})
0% P + AMF	3.17 ± 0.10 c	0.30 ± 0.09 c	10.64 ± 1.14 b	1.56 ± 0.10 c	3.14 ± 0.17 c
12.5% P + AMF	7.75 ± 0.26 b	0.64 ± 0.06 b	14.29 ± 1.76 b	1.82 ± 0.06 c	6.36 ± 1.81 b
25% P + AMF	13.99 ± 0.29 a	1.14 ± 0.08 a	19.18 ± 1.69 a	10.29 ± 1.58 a	9.14 ± 1.39 a
50% P + AMF	13.00 ± 0.29 a	1.24 ± 0.08 a	17.64 ± 1.39 a	6.53 ± 1.19 a	9.81 ± 1.82 a
100% P + AMF	16.31 ± 0.42 a	1.34 ± 0.05 a	18.37 ± 1.12 a	9.06 ± 1.35 a	9.07 ± 1.28 a
100% P − AMF	11.88 ± 0.87 b	1.02 ± 0.02 b	16.40 ± 1.47 a	4.88 ± 1.12 b	8.79 ± 1.09 a
Significance	**	**	**	**	**
Mean	11.01	0.95	16.09	5.69	7.72
CV [2] (%)	12.66	13.03	12.39	18.63	14.61
Treatments	**FKC (mg/g DM^{-1})**	**CCAC (mg/g DM^{-1})**	**APCAC (mg/g DM^{-1})**	**CMGC (mg/g DM^{-1})**	**FMGC (mg/g DM^{-1})**
0% P + AMF	73.54 ± 7.10 b	0.74 ± 0.02 c	26.48 ± 2.46 b	0.56 ± 0.01 b	0.67 ± 0.02 b
12.5% P + AMF	62.97 ± 6.25 c	1.92 ± 0.08 b	25.47 ± 2.43 b	0.69 ± 0.02 b	1.37 ± 0.77 b
25% P + AMF	180.97 ± 9.97 a	3.08 ± 0.29 a	51.91 ± 3.89 a	1.35 ± 0.09 a	6.12 ± 0.81 a
50% P + AMF	137.24 ± 8.79 a	3.16 ± 0.58 a	41.96 ± 3.78 a	1.34 ± 0.10 a	3.64 ± 0.54 a
100% P + AMF	153.42 ± 9.44 a	2.45 ± 0.43 a	45.36 ± 3.15 a	1.34 ± 0.11 a	5.62 ± 0.69 a
100% P − AMF	60.40 ± 5.04 c	1.74 ± 0.09 b	37.61 ± 2.97 a	0.95 ± 0.07 a	3.63 ± 0.17 a
Significance	**	**	**	**	**
Mean	111.42	2.18	38.13	1.04	3.51
CV (%)	19.39	16.17	10.82	12.13	13.56

Data are presented as mean ± standard deviation. Means followed by the same letter in the column did not differ between each other according to Duncan's test ($p \leq 0.05$). **: significant at the 1% probability level ($p < 0.01$). NS: treatment effect not significant ($p > 0.05$). [1] CNC: crown nitrogen content; CPC: crown phosphorus content; APPC: aerial part phosphorus content; FPC: fruit phosphorus content; CKC: crown potassium content; FKC: fruit potassium content; CCAC: crown calcium content; APCAC: aerial part calcium content; CMGC: crown magnesium content; FMGC: fruit magnesium content. [2] CV: coefficient of variation.

Plants treated with 25% P + AMF showed nutritional compositions of the plant organs (mg/g DM^{-1}) of 15%, 11%, 53%, 67%, and 43% more CNC, CPC, FPC, FKC, and CCAC, respectively, in relation to plants grown with 100% P − AMF (Table 7).

3.6. Multivariate Analysis

The PCA results showed that the first four PCs can be used to study the relationships among fruit production and quality, root system morphology, and the nutritional composition of strawberry organs grown with AMF and reduced P supply, since the eigenvalues were greater than 1 (Table 8). The first two PCs had a cumulative proportion of 59.38% (Table 8), with the first PC explaining 37.7% of the total variance and the second PC explaining 21.7% (Figure 5).

Table 8. Principal components (PC), autovalues (λi), proportion of variance (PV), and accumulated proportion of variance (APV) of the components.

PC	λ_i	PV (%)	APV (%)
1	6.027421230	37.6713827	37.67138
2	3.473581349	21.7098834	59.38127
3	1.844958177	11.5309886	70.91225
4	1.315524645	08.2220290	79.13428

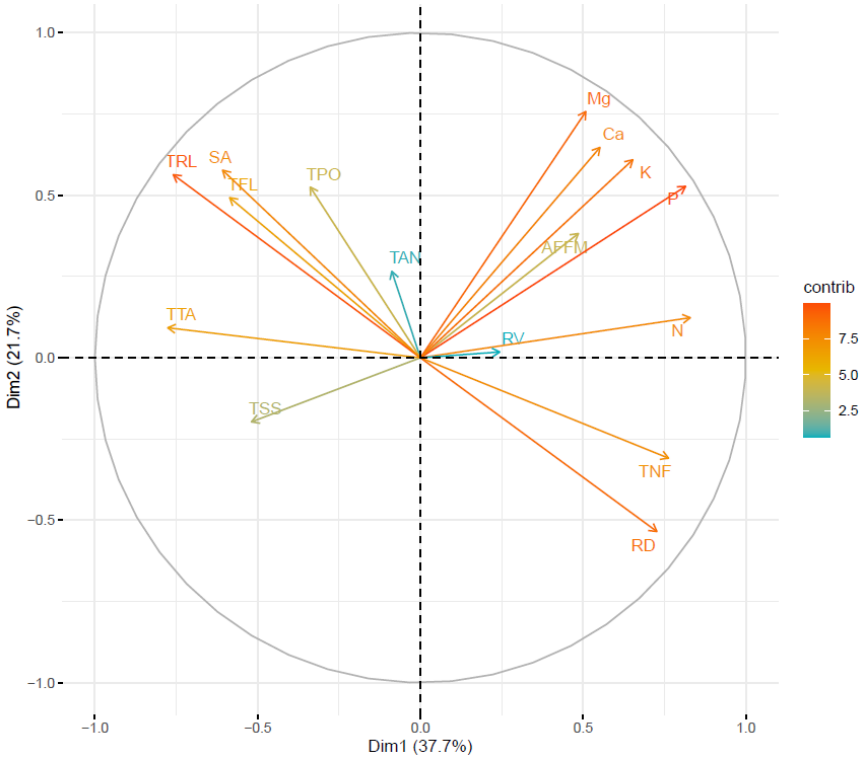

Figure 5. Principal component analysis with the contribution percentage of the attributes analyzed in the study. TNF: total number of fruits; AFFM: AFFM: average fresh fruit mass; TSS: total soluble solids; TTA: total titratable acidity; TAN: total anthocyanins; TFL: total flavonoids; TPO: total polyphenols; TRL: total root length; SA: superficial area; RV: root volume; RD: root diameter; N: nitrogen content; P: phosphorus content; K: potassium content; Ca: calcium content; Mg: magnesium content.

When we analyzed all the attributes together (fruit production and quality, root system morphology, and organ nutritional composition), we found that the treatments behaved dissimilarly, forming three groups. One of them brought together the control treatment (100% P − AMF) and the treatments with the two highest doses of P in association with AMF (Figure 6), strengthening the results in terms of fruit quality (Table 5) and the performance of the plants' root systems (Table 6). Another group included the treatments with the two lowest doses of P (0% and 12.5%) in the presence (+) of AMF (Figure 6), and this mainly corroborated the results of the nutritional composition of the organs (Table 7). Finally, the use of 25% P with AMF was not grouped with any other treatment (Figure 6), and this showed its better performance, in terms of balance and in terms of the possibility of reducing the P supply by 75% conditioned on the use of AMF, without harming fruit production (Table 4) and quality (Table 5), root development (Table 6), or nutrient accumulation in plant organs (Table 7).

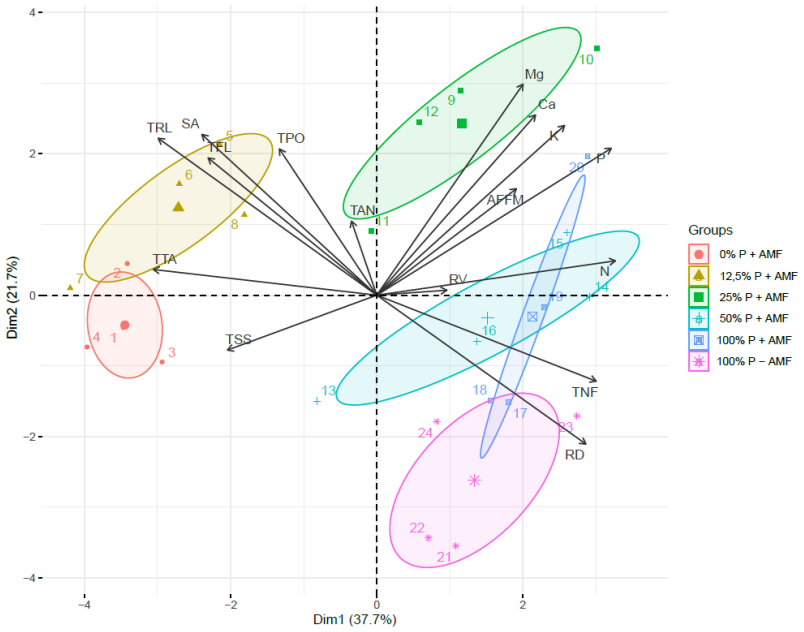

Figure 6. Clustering relationship, through principal component analysis, among six treatments analyzed in the study. TNF: total number of fruits; AFFM: average fresh fruit mass; TSS: total soluble solids; TTA: total titratable acidity; TAN: total anthocyanins; TFL: total flavonoids; TPO: total polyphenols; TRL: total root length; SA: superficial area; RV: root volume; RD: root diameter; N: nitrogen content; P: phosphorus content; K: potassium content; Ca: calcium content; Mg: magnesium content.

This same grouping of treatments was seen in the dendrogram generated using the single linkage method (Figure 7), which strengthens our results that the combination of using AFM with only 25% P in the nutrient solution stands out. These dissimilarities among the six treatments, illustrated by dendrogram (Figure 7), had an adjustment to the data distance matrix calculated by the CCC of 95%, which indicated grouping adequacy.

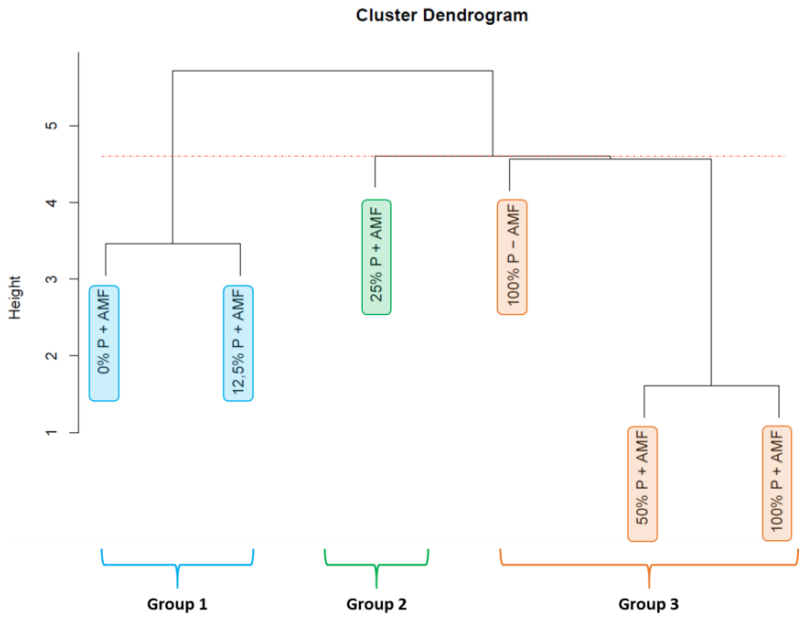

Figure 7. Dendrogram created using the single linkage method (nearest neighbor) of the six treatments, based on yield, fruit quality, root system morphology, and nutritional composition of organs in strawberry plants.

4. Discussion

The response of the strawberry plants to AMF colonization depended on the cultivar used [36] and, in our case, also on the P supply during cultivation. There were significant differences among the different treatments, and the 100% phosphorus supply with (100% P + AMF) and without (100% P − AMF) mycorrhizae resulted in the lowest AMF colonization on the strawberry plants (Figure 3). Our research showed the same result as the plants in the Fu et al. [37] trial, as they had no AMF contamination in the non-mycorrhizal control (Figure 3).

The low level of mycorrhizal colonization (23%) observed in the 100% P + AMF treatment indicated that excessive P use reduced the association with AMF, equating it with the 100% P − AMF treatment (Figure 3). Exogenous P supply suppressed arbuscule development and limited intraradical colonization [38]. The literature reports that host defense mechanisms participate in this reduction in intraradical colonization in plants cultivated with high levels of P. Quantitative trait locus (QTL) analyses have indicated at least two genetic loci related to the defense and metabolism of the cell wall of plant host [39]. It is believed that a resistance mechanism to AMF was selected in domesticated plants in agroecosystems with high input consumption, which led to reduced activity in the mycorrhizal phosphate absorption pathway [40].

The treatments with the lowest P (0% P + AMF and 12.5% P + AMF) inputs and the control treatment (100% P − AMF) produced the groups with the lowest grams of AFFM. This is due to nutrients being essential elements for plant development and fruiting (Table 4). In the case of strawberry plants, non-fertilization of the plants leads to low fruiting values [41]. Previous studies have demonstrated the dynamics of nutrient uptake by strawberry plants grown soilless culture [18] and the benefits of AMF in strawberry cultivation [1,36]. AMF form a network of filaments around the roots, which allows the host root system to further explore the growth medium. This enhances the roots' ability to acquire nutrients such as nitrogen (N), P, copper (Cu), and zinc (Zn) [42,43], which improves crop yields and reduces the need for fertilizers [44]. One of the most notable benefits of AMF is the increase in P consumption by the plant. Briefly, the P acquisition strategy comprises three phases: (1) P assimilation through AMF hyphae; (2) movement along the hyphae from the outside to the inside of the mycelium; and (3) exchange of P to root cortical cells [45]. In addition to nutritional benefits, the presence of mycorrhizal hyphae on plant roots increases aeration of the growing medium, improves water retention, and suppresses disease-causing phytopathogens [46].

Although the 0% P + AMF (0 mg.plant^{-1}) and 12.5% P + AMF (34 mg.plant^{-1}) treatments reduced TP, the use of 25% P + AMF (69 mg.plant^{-1}) and 50% P + AMF (137 mg.plant^{-1}) resulted in a balance between the reduction in P supply and the strawberry yield (Table 4). These responses symbolize the energy cost of establishing and maintaining the symbiosis. The source of C needed for mycorrhizal maintenance comes only from the metabolism of plants [47]. Thus, in environments where the availability of P is not sufficient to maintain the plant's physiological processes, the plant symbiont reduces the C supply to the AMF, and this limits the association [48]. Therefore, we report that a reduction of up to 75% in the P supply represents a balance between AMF and strawberry plants in the soilless cultivation system, with adequate distribution of carbohydrates to the microorganisms and no damage to fruit production. Although strawberry plants have a low P requirement [18], a deficiency of this nutrient reduces flowering and fruit sets and causes fruit discoloration [49]. However, inoculation with AMF increases photosynthesis [50], induces early flowering and fruiting [51], and increases the content of functional compounds in strawberries [1]. This suggests that AMF can alleviate the negative effects of lower P supply.

The treatments had no significant effect on strawberry quality, and TSS were not influenced by treatments in our research. However, as fruit maturity progresses and sugar content increases, TSS will also increase [52]. Yu et al. [53] stated that substrates had no

significant effect on strawberry quality, but the nutrient solution and substrate coupling had a significant effect on it.

The antioxidant activity of strawberry fruits is directly related to their phenolic compounds (flavonoids and anthocyanins). The main group of phenolic compounds in strawberry fruits consists of flavonoids, which have been shown to have antioxidant and anticancer properties [54,55]. Plants grown with AMF and less phosphorus (0% P + AMF, 12.5% P + AMF, and 25% P + AMF) produced fruit with higher amounts of TFL and TPO (Table 5). Phenolic compounds, especially flavonols, are potent antioxidants compared to traditional vitamins [56]. Strawberries have high contents of flavonoid pigments called anthocyanins [57]. Although, in our research, there were no significant differences in total anthocyanins among treatments (Table 5), they contribute significantly to the total antioxidant activity of this fruit [54,58]. The increase in TFL and TPO levels in fruit produced by plants cultivated with AMF (Table 5) is linked to a defense response by the plant symbiont to mycorrhizal colonization of the root system (Figures 3 and 4). This stress factor induces the plant's secondary metabolism, which is responsible for enhancing the synthesis of phytochemicals. Consumer demand for foods rich in secondary metabolites has led to changes in the standards of product supply in commercial networks. This search for food quality can be seen as a demand for strawberry producers, who can use mycorrhizal biotechnology as a strategy to improve the fruit's phytochemical content.

In addition to fruit yield and quality, the interface between P reduction and the use of AMF improved the development of the plants' root system. During the symbiotic process, the host root releases strigolactone, a phytohormone that stimulates the branching of hyphae. In turn, the AMF release molecular signals (lipoquito-oligosaccharide) which induce the activation of "symbiosis genes" in the plant, and this allows for recognition between partners, the formation of the hyphae network [59], and changes to the root architecture [1]. These changes include a greater number of branches, greater length, and greater volume, as was observed when the plants were treated with 25% P + MFA (69 mg.plant^{-1}) (Table 6). A more profuse root system, associated with the network of hyphae, provides the host with greater efficiency in capturing water and nutrients, which also explains our results regarding fruit yield (Table 4) and quality (Table 5), as well as the nutritional composition of plant organs (Table 7). The architectural benefits of the root systems of mycorrhized plants favor photosynthetic efficiency [50], the activity of enzymes linked to the defense system [60], and increased nutritional content [61].

By regulating the absorption and recycling of nutrients, AMF can modify the growth and development of host plants [62]. The treatment that obtained the highest value of elements in most of the plant organs was 25% P + AMF. Mycorrhizal biotechnology, combined with the use of only 25% P in the nutrient solution, increased the content of N, P, K, Ca, and Mg in the crown, aerial part, and/or fruit (Table 7), indicating that the symbiosis affects the allocation of nutrients in strawberry organs. AFM fungi play a crucial role in nutrient uptake by plants, resulting in more efficient nutrient acquisition [63,64]. Some authors have observed that inoculation with AMF increases Ca uptake in some plants [37]. In our case, the highest Ca uptake values were generated in the plants inoculated with AMF and the highest P inputs (25% P + AMF, 50% P + AMF, and 100% P + AMF) (Table 7). AMF have been reported to be effective in the uptake of Zn, Cu, Mn, Fe, Ca, K, and N, in addition to playing a significant role in phosphorus uptake [65–67].

The mycorrhizal community used here came from soil under strawberry cultivation with 'Camarosa' [20], the same cultivar tested in our study. This indicated that the six fungal species in the community (see Section 2.2) had a greater affinity with 'Camarosa'. Despite reports of preferences for plant symbionts [68], AMF associate with a wide range of plants, and this highlights the lack of host specificity due to effector proteins secreted by the microorganisms [69], which manipulate host cells to facilitate successful infection. This alters the structure of the host and also modifies the plant's metabolism [70].

Only one mycorrhizal inoculant is commercially available in Brazil, made up of *Rhizophagus intraradices* (formerly *Glomus intraradices*). In general, commercial inoculants are

monospecific, and the species they contain may not be adapted to the conditions of the agroecosystem. As the use of fungal species that are compatible with the plant partner increases arbuscular mycorrhiza [71], we would point out that our multi-specific on-farm inoculant came from soil adapted to strawberry cultivation [20]. The application of native AMF improves plant development because indigenous AMF are genetically and physiologically adapted to their native host [72]. In strawberry plants, the association between the native mycorrhizal community and biochar intensified mycorrhizal colonization and increased root development [1].

The practice of conventional agriculture hinders the symbiosis and effectiveness of AMF. High P concentration, excessive use of agrochemicals, and inadequate management of inoculants (e.g., AMF–host affinity, strain-growth medium compatibility) are some of the challenges that horticulturists face and that can negatively compromise the activity of AMF [73]. This indicates that mycorrhizal behavior depends greatly on fluctuations in the agroecosystem [74]. Furthermore, the lack of knowledge about mycorrhizal biotechnology on the part of horticulturists and the lack of commercial inoculants (although on-farm production is a viable alternative) limit the adoption of this bio-tool in commercial strawberry crops. However, AMF become inevitable to sustainability in agriculture [73]. The successful application of AMF in horticultural agroecosystems demands extensive management adjustments, knowledge about the effects of agricultural changes, and mycoengineering of the associated microbiota [75].

As a successful bioinoculant aimed at sustainable agriculture, AMF contributes to greater production of quality food with lower environmental impacts [74]. Herein, we showed that the use of a multi-species on-farm inoculant made up of six fungal species native to the soil of a strawberry-growing reference site [20] made it possible to reduce the P supply by up to 75% during the first production cycle. This represents a reduction in production and environmental costs in agroecosystems under strawberry cultivation. This characteristic of AMF as a biofertilizer allows these microorganisms to be used as a bio-tool for the transition to sustainable horticultural systems, with a reduction in the application of chemical inputs and, therefore, with fewer negative impacts on the environment.

5. Conclusions

The use of a multi-species on-farm inoculant based on AMF associated with a reduction in P supply in the nutrient solution modified the horticultural potential of strawberry plants of the 'Camarosa' cultivar. These results provide an important reference for the quantity and quality characteristics of strawberry fruits, the morphology of the root system of strawberry plants, and the macronutrients of plant organs of strawberry plants under different conditions of decreased P inputs with mycorrhizal-colonized roots. The addition of AMF to the growing substrate made it possible to reduce the P amount in the nutrient solution by 75% without compromising the fruit yield. Furthermore, this combination improved the phytochemical quality of strawberries, the morphology of the root system, and the accumulation of nutrients in plant organs. We conclude that the combined addition of AMF and 25% P in the cultivation strawberry benefits the primary macronutrient content in plant organs. This allows us to confirm the biofertilizing nature of AMF, which could support the incorporation of this bio-tool in strawberry cultivation in transition processes towards more sustainable systems.

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