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Phosphorus Dynamics in Stressed Soil Systems: Is There a Chemical and Biological Compensating Effect?

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Abstract: Here, we hypothesized the occurrence of a compensatory relationship between the application of P and different microbial communities in the soil, specifically in relation to the chemical and biological effects in the soil–plant–microorganisms' interaction. We aimed to evaluate the plant–microbiota responses in plants grown in soils hosting distinct microbial communities and rates of P availability. Two experiments were carried out in a greenhouse. The first experiment evaluated four manipulated soil microbiome compositions, four P rates, and two plant species. Manipulated soil systems were obtained by the following: (i) autoclaving soil for 1 h at 121 °C (AS); (ii) inoculating AS with soil suspension dilution (AS + 10^{-3}); (iii) heating natural soil at 80 °C for 1 h (NH80); or (iv) using natural soil (NS) without manipulation. The P rates added were 0, 20, 40, and 60 mg kg^{-1}, and the two plant species tested were grass (brachiaria) and leguminous (crotalaria). Inorganic labile P (P_{AER}), microbial P (P_{MIC}), acid phosphatase activity (AP_{ASE}), and shoot P uptake (P_{UPT}) were assessed for each system. Brachiaria presented a compensatory effect for P_{UPT}, whereby the addition of P under conditions of low microbial community enhanced P absorption capacity from the soil. However, in a system characterized by low P input, the increase in the soil biodiversity was insufficient to enhance brachiaria P_{UPT}. Likewise, crotalaria showed a higher P_{UPT} under high P application and low microbial community. The second experiment used three manipulated microbiome compositions: AS + 10^{-3}; NH80; and NS and three P rates added: 0, 20, and 40 mg kg^{-1}. In addition, two treatments were set: without and with mycorrhiza inoculation. Brachiaria showed an increase in the P_{UPT} under low microbial communities (AS + 10^{-3}; NH80) with P addition (20 and 40 mg kg^{-1} of P), but no mycorrhization was observed. In the undisturbed microbial community (NS), under no P input (0 mg kg^{-1} of P), brachiaria showed low mycorrhization and low P_{UPT}. Finally, NS and the recommended P input (40 mg kg^{-1} of P) represented a balance between chemical and biological fertility, promoting the equilibrium between mycorrhization and P_{UPT}.

Keywords: *Urochloa brizantha* cv. Marandu; *Crotalaria juncea* L.; *Rhizophagus clarus*; labile P; acid phosphatase activity; microbial P; shoot P uptake

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1. Introduction

In highly weathered tropical soils, phosphorus (P), a macronutrient required for plant development, is strongly adsorbed on the surface of sesquioxides, such as aluminum and iron oxides, which reduces its availability to the plants [1]. The lack of available P to

the plants induces plant stress, affecting both the morphological and physiological plant aspects, which may be evidenced by some visual symptoms, such as a purplish color in the shoot.

To supply nutrients to plants in an agroecosystem, it is necessary to consider the fertility concept, which encompasses the physical, chemical, and biological components of the soil, all of which are interconnected and influence each other. However, in general, agricultural practices tend to overemphasize one factor at the expense of others. For instance, one of the most used P fertilizers by farmers (i.e., triple superphosphate—TSP) is often applied into the soil based only on the chemical traits [2], but when TSP is applied, the adsorption process rapidly diminishes P availability to the plants [3], reducing P mobility in the soil and restricting the contact between phosphate and roots for absorption [4], increasing the legacy P in the soil [5]. Furthermore, an important factor to consider is that apatite rocks, the main mineral source for P fertilizer, are a finite resource and must be used in a sustainable way to ensure food security for both the present and the future.

In this context, the biological fertility aspects become important strategies to improve soil quality. Microorganisms play a significant role in the soil P cycle, and therefore, they mediate the availability of P for plants [6]. Many microorganisms are responsible for increasing P availability to the plants through various processes such as mineralization of P from organic forms and/or solubilization from inorganic stable forms of P present in the soil [7].

When inorganic P (Pi) availability in the soil is low, plants can use the organic P (Po) through mineralization by enzymes, such as phosphatase present in the rhizosphere, increasing inorganic P availability for plants [8,9]. The phosphatase activity is strongly influenced by the soil pH, where neutral phosphomonoesterase may be found in a pH optimum ranging from 4.6 to 7.0; acid phosphatase with a pH optimum of 4.8; and alkaline phosphatase in a pH optimum of 11.0 [10].

Another biological strategy is the use of cover crops to improve several fertility soil aspects. Distinct crop species, such as brachiaria (*Urochloa brizantha* cv. Marandu) and crotalaria (*Crotalaria juncea* L.), have been used to improve the soil's physical, chemical, and biological attributes. Crops that belong to distinct botanical families exhibit diverse morphological and physiological features, providing different benefits to an agroecosystem, including nutrient availability, such as N and P.

A further strategy is the addition of beneficial microorganisms into the soil, known as bioinputs, to enhance the soil biological functionality and hence improve soil quality. Therefore, the inoculation of mycorrhiza may help in the soil P usage. Once mycorrhiza colonization occurs in the plant root system, a symbiosis may be established, where the fungal hyphae may increase the soil volume exploited by the plant root system, and hence, the fungi provide nutrients with low mobility in the soil, such as P, to the plants. The plants, in return, provide energy though C compounds to the fungi.

Therefore, the soil microbiome community presents important benefit roles. However, the microbial community might cause stress conditions to the plants, either by the presence of harmful organisms that may compete with the plant for resources or cause certain diseases or the absence of beneficial organisms. This underscores the importance of the soil microbial composition in an agroecosystem. Based on the microbial community present in the soil, plants have strategies for selecting organisms in the rhizosphere, which can have positive, negative, or neutral effects on the plant [11], either to minimize the undesired or potential beneficial effects of the microbial community on the crops.

Though the management practices are implemented separately in agroecosystems, the physical, chemical, and biological features interact and can either enhance or harm each other. Consequently, P use efficiency is dependent on soil fertility, and the combination of efficient chemical and biological management practices can be a viable strategy for soil exploitation and plant P uptake, and one point may compensate the other, once it is under an unbalanced condition [12]. Although some mechanisms involving the regulation of P uptake have been studied, further research is needed to better understand the interaction

mechanisms between chemical and biological factors that affect P uptake and utilization in plants [13]. Thus, strategies encompassing plants features, soils and rhizosphere microbial diversity, and fertilizers' application should be coordinated to ensure the sustainability of the agroecosystem [14].

The hypothesis tested here was the occurrence of a compensatory relationship between the application of P and different microbial communities in the soil, specifically in relation to chemical and biological effects in the soil–plant–microorganisms' interaction. The objective of this work was to evaluate the plant–microbiota responses in the plants grown in soils hosting distinct microbial communities and the rates of P availability.

2. Results

2.1. Experiment 1: Manipulation of Soil Microbiome and P Doses Application

In terms of chemical fertility, the autoclaving and the heating methods used to obtain the treatments AS and NH80, respectively, changed the concentrations of P_{AER}, Fe, and Mn compared to the NS (Table 1). The dilution–extinction methods had no effect on the other chemical characteristics.

Table 1. Soil chemical parameters: P_{AER}, Fe, and Mn from soil samples submitted to soil microbial manipulation and P rate application.

Treatments	P_{AER} (mg kg^{-1})			
	P (mg kg^{-1})			
	0	20	40	60
AS	0.86	2.84	2.63	6.11
NH80	2.95	4.77	6.65	8.10
NS	3.92	5.04	6.87	8.10
	Fe (mg dm^{-3})			
	P (mg kg^{-1})			
	0	20	40	60
AS	100	105	170	122
NH80	79	80	74	77
NS	77	79	78	85
	Mn (mg dm^{-3})			
	P (mg kg^{-1})			
	0	20	40	60
AS	32.8	33.8	26.9	31.5
NH80	7.0	7.8	7.6	5.8
NS	5.8	5.5	6.4	5.9

- + Color scale was used for all sets of data for each variable. Treatment abbreviations are as follows: sterilized soil by autoclaving twice (121 °C, 103 kPa, 1 h) without soil dilution inoculation (AS); soil heated at 80 °C for 1 h (NH80); and natural soil without manipulation (NS). P fertilizer was applied as triple superphosphate at the following rates: 0; 20; 40; and 60 mg kg^{-1} of P. P_{AER}: inorganic labile P extracted using anion exchange resin and determined using colorimetric assay; Fe and Mn extracted using diethylenetriaminepentaacetic acid (DTPA) and determined using atomic absorption spectrophotometry.

Regarding P_{AER}, the P application linearly increased the P content in the soil for AS, NH80, and NS, where low P application results in lower P_{AER}, while higher P application doses resulted in high P_{AER}. The autoclaving procedure decreased the P_{AER} compared to that in NS within the same P dosage application, but no significant changes were observed for NH80 compared to that for NS (Table 1).

Overall, the P application had no effect on the levels of the micronutrients Fe and Mn in the soil; however, the autoclaving process raised the levels compared to that in NS, and no changes were observed in NH80 when compared with that in NS (Table 1).

Regarding the biological features, in general, the lowest values of AP_{ASE} were observed under autoclaved soil (AS), followed by AS + 10^{-3}, under P application (20, 40, and 60 mg kg^{-1}). On the other hand, the highest values were observed under NH80 and NS for all the P doses tested (Table 2). The bacterial and fungal abundance (qPCR) slightly

changed along the treatments. For fungi, in general, the highest values of abundance were observed for AS and AS + 10^{-3} treatment (Table 2).

Table 2. Biological parameters: AP_{ASE}, qPCR (bacteria and fungi) from soil samples submitted to soil microbial manipulation, and P rate application.

Treatments	AP_{ASE} (mg kg^{-1} h^{-1})							
	P (mg kg^{-1})							
	0		20		40		60	
AS	317	dA	143	cBC	168	dB	133	cBC
AS + 10^{-3}	409	cA	212	bBC	319	cAB	250	bB
NH80	585	bBC	645	aB	531	bC	745	aA
NS	726	a ^{ns}	753	a ^{ns}	736	a ^{ns}	682	a ^{ns}
	Gene abundance (log g^{-1})—16S—bacteria							
	P (mg kg^{-1})							
	0		20		40		60	
AS	10.15	bB	10.09	cC	10.25	bA	10.21	abAB
AS + 10^{-3}	10.27	aB	10.33	aA	10.33	aA	10.19	bC
NH80	10.18	bB	10.18	bB	10.19	cAB	10.22	abA
NS	10.27	aB	10.34	aA	10.32	aAB	10.26	aC
	Gene abundance (log g^{-1})—ITS—fungi							
	P (mg kg^{-1})							
	0		20		40		60	
AS	8.12	bD	8.48	bC	8.71	aB	8.87	aA
AS + 10^{-3}	8.44	aB	8.70	aA	8.69	aA	8.30	bB
NH80	7.76	bc ^{ns}	7.83	d ^{ns}	7.84	b ^{ns}	7.81	c ^{ns}
NS	7.90	b ^{ns}	7.99	c ^{ns}	7.98	b ^{ns}	7.85	c ^{ns}

Color scale was used for all sets of data for each variable. Different uppercase letters within the same row show significant differences among P doses by Student's *t*-test ($p \leq 0.05$). Different lowercase letters within the same column show significant differences among microbial communities by Student's *t*-test ($p \leq 0.05$). ^{ns} means non-significant difference by Student's *t*-test ($p \leq 0.05$). Treatment abbreviations are as follows: sterilized soil by autoclaving twice (121 °C, 103 kPa, 1 h) without dilution inoculation (AS); soil heated at 80 °C for 1 h (NH80); and natural soil without manipulation (NS). P fertilizer was applied as triple superphosphate at the following rates: 0; 20; 40; and 60 mg kg^{-1} of P.

2.2. P Dynamics after Plant Cultivation under Microbial Manipulation and P Fertilizer Rates Application

Using similar microbial communities and P doses, principal component analysis (PCA) showed a significant difference in the responses of crotalaria and brachiaria to the evaluated variables, with a data explanation of 67% (PC1 45% and PC2 22%). The second component separated the two crops. Brachiaria was closely associated with high microbial and plant features (AP_{ASE}, P_{MIC}, and P_{UPT}), while crotalaria was more related to high P_{AER} (Figure 1). Due to these differences, results for each crop will be presented separately.

For brachiaria, the interaction between P levels and the levels of microbial community was observed for all the evaluated variables (P_{AER}, AP_{ASE}, P_{MIC}, and P_{UPT}). The highest availability of P (P_{AER}) after cropping with brachiaria was observed under conditions with high levels of microbial community (NS and NH80) and high rates of P applied (40 and 60 mg kg^{-1}), represented by the green color in Table 3. In contrast, the treatments AS + 10^{-3} and AS, under no or low P application (0 and 20 mg kg^{-1}), showed the lowest available P after cropping, represented by the red color in Table 3.

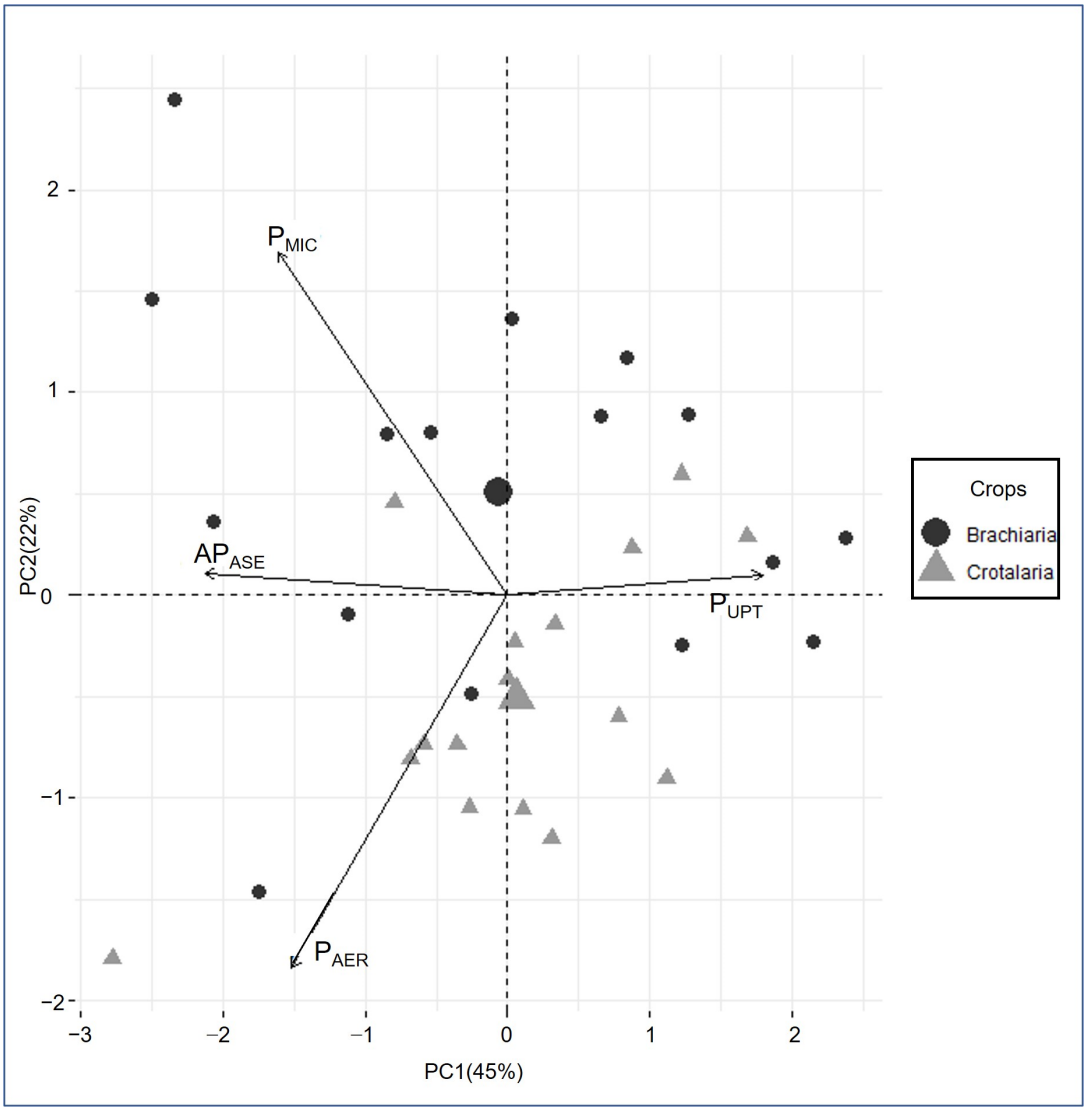

Figure 1. Principal component analysis (PCA) of P_{AER}, AP_{ASE}, P_{MIC}, and P_{UPT} from samples taken after cropping with brachiaria and crotalaria in soils subjected to soil microbial manipulation and P rate application. Treatment abbreviations are as follows: sterilized soil by autoclaving twice (121 °C, 103 kPa, 1 h) without soil dilution inoculation (AS); autoclaved soil followed by re-inoculation with dilution from the natural soil (10% w/v of natural soil) (AS + 10^{-3}); soil heated at 80 °C for 1 h (NH80); and natural soil without manipulation (NS). P fertilizer was applied as triple superphosphate at the following rates: 0; 20; 40; and 60 mg kg^{-1} of P. P_{AER}: inorganic labile P extracted using anion exchange resin; AP_{ASE}: acid phosphatase activity; P_{MIC}: microbial P; P_{UPT}: up taken P on shoot.

When brachiaria was grown under NS and NH80 and high P application (40 and 60 mg kg^{-1}), the maximum activity of AP_{ASE} was observed. Conversely, low microbial community levels (AS + 10^{-3} and AS) and high P application (40 and 60 mg kg^{-1}) were associated with the lowest AP_{ASE} activity (Table 3).

High P immobilization in the microbial biomass (P_{MIC}) was observed under high levels of microbial community (NS) but with low and intermediate levels of P application (0 mg kg^{-1}, followed by 40 and 20 mg kg^{-1}). Furthermore, the P_{MIC} values were decreased with the intermediate and high P rates (20 mg kg^{-1}, then 40 and 60 mg kg^{-1}) under the manipulated microbial community (NH80, then AS + 10^{-3} and AS) (Table 3).

Table 3. P_{AER}, AP_{ASE}, P_{MIC}, and P_{UPT} from samples taken after cropping with brachiaria in a soil subjected to soil microbial manipulation and P rate application.

Treatment	P_{AER} (mg kg^{-1})							
	P (mg kg^{-1})							
	0		20		40		60	
AS	0.76	cD	2.32	bC	4.62	cB	7.30	bA
AS + 10^{-3}	1.28	bC	2.78	bB	6.22	cA	5.62	cA
NH80	4.32	aD	6.68	aC	14.70	aA	10.98	aB
NS	4.94	aB	8.12	aA	8.94	bA	10.20	aA
	AP_{ASE} (mg kg^{-1} h^{-1})							
	P (mg kg^{-1})							
	0		20		40		60	
AS	509	bA	235	bB	250	cB	238	bB
AS + 10^{-3}	473	bA	225	bC	300	bB	279	bB
NH80	639	aB	658	aB	744	aA	774	aA
NS	678	aA	210	bB	740	aA	752	aA
	P_{MIC} (mg kg^{-1})							
	P (mg kg^{-1})							
	0		20		40		60	
AS	7.0	cA	10.8	aA	6.3	bA	6.7	cA
AS + 10^{-3}	14.1	bA	15.2	aA	5.0	bB	8.9	bcAB
NH80	13.3	bA	2.7	bB	7.5	bAB	15.2	abA
NS	34.0	aA	22.2	aAB	30.2	aAB	19.8	aB
	P_{UPT} (µg pot^{-1})							
	P (mg kg^{-1})							
	0		20		40		60	
AS	410	aC	533	aB	927	aA	1272	aA
AS + 10^{-3}	230	bD	429	aC	662	aB	1469	aA
NH80	146	cD	329	bB	230	bC	728	bA
NS	63	dC	112	cB	199	bA	196	cA

- + Color scale was used for all sets of data for each variable. Different uppercase letters within the same row show significant differences among P doses by Student's *t*-test ($p \leq 0.05$). Different lowercase letters within the same column show significant difference among microbial community by Student's *t*-test ($p \leq 0.05$). Treatment abbreviations are as follows: sterilized soil by autoclaving twice (121 °C, 103 kPa, 1 h) without dilution inoculation (AS); soil heated at 80 °C for 1 h (NH80); and natural soil without manipulation (NS). P fertilizer was applied as triple superphosphate at the following rates: 0; 20; 40; and 60 mg kg^{-1} of P. P_{AER}: inorganic labile P extracted using anion exchange resin; AP_{ASE}: acid phosphatase activity; P_{MIC}: microbial P; P_{UPT}: up taken P on shoot.

Higher P uptake was observed when brachiaria was cultivated under high P rates (60 mg kg^{-1}, followed by 40 mg kg^{-1}) and low levels of microbial community (AS + 10^{-3} and AS). In contrast, a reduction in P uptake was observed when brachiaria was cultivated under no or low P application (0 and 20 mg kg^{-1}) with high levels of microbial communities (NS, followed by NH80) (Table 3).

For crotalaria, the interaction between P levels and the levels of microbial community was observed for P_{AER} and AP_{ASE}, but not for P_{MIC} and P_{UPT}. For this reason, the interaction values for P_{MIC} and P_{UPT} do not display a color scale, instead the means for each factor are displayed (Table 4).

The conditions with the highest P_{AER} were no P application (0 mg kg^{-1}) and high levels of microbial community (NS treatment), as represented by the green color in Table 4. Moreover, the lowest P_{AER} was observed when crotalaria was grown under low level of microbial community (AS) and an intermediary rate of P application (40 mg kg^{-1}), as represented by the red color in Table 4.

Table 4. P_{AER}, AP_{ASE}, P_{MIC}, and P_{UPT} from samples taken after cropping with crotalaria in a soil subjected to soil microbial manipulation and P rate application.

Treatments	P (mg kg^{-1}) 0		P (mg kg^{-1}) 20		P (mg kg^{-1}) 40		P (mg kg^{-1}) 60		Mean
P_{AER} (mg kg^{-1})									
AS	6.75	dA	8.61	cA	0.52	bB	4.02	bA	
AS + 10^{-3}	10.00	bA	10.10	aA	2.86	bC	7.42	aB	
NH80	9.63	cA	9.03	bAB	7.13	aB	8.41	aAB	
NS	21.80	aA	9.63	bB	4.27	aC	6.19	aB	
AP_{ASE} (mg kg^{-1} h^{-1})									
AS	382	abA	89	cC	330	bAB	179	bB	
AS + 10^{-3}	292	bA	285	bA	377	bA	301	abA	
NH80	560	aA	591	aA	472	abA	649	aA	
NS	580	aAB	525	aAB	771	aA	392	aB	
P_{MIC} (mg kg^{-1})									
AS	6.74	ns	3.15		3.71		7.13		5.18 b
AS + 10^{-3}	3.97		2.34		4.04		3.64		3.50 b
NH80	5.34		4.82		4.98		3.20		4.59 b
NS	18.60		2.49		8.66		6.40		9.04 a
Mean	8.66	A	3.20	B	5.35	AB	5.09	AB	
P_{UPT} (µg pot^{-1})									
AS	162	ns	286		302		667		354 a
AS + 10^{-3}	138		201		330		408		269 bc
NH80	162		221		259		379		255 c
NS	119		257		259		313		237 c
Mean	145	C	241	B	287	B	442	A	

Color scale was used for all sets of data for each variable. Different uppercase letters within the same row show significant differences among P doses by Student's *t*-test ($p \leq 0.05$). Different lowercase letters within the same column show significant difference among microbial community by Student's *t*-test ($p \leq 0.05$). ^{ns} means non-significant difference by Student's t-test ($p \leq 0.05$). Treatment abbreviations are as follows: sterilized soil by autoclaving twice (121 °C, 103 kPa, 1 h) without soil dilution inoculation (AS); soil heated at 80 °C for 1 h (NH80); and natural soil without manipulation (NS). P fertilizer was applied as triple superphosphate at the following rates: 0; 20; 40; and 60 mg kg^{-1} of P. P_{AER}: inorganic labile P extracted using anion exchange resin; AP_{ASE}: acid phosphatase activity; P_{MIC}: microbial P; P_{UPT}: up taken P on shoot.

Crotalaria cultivation resulted in the highest activity for AP_{ASE} when the microbial community (NS, followed by NH80) and P application (40 and 60 mg kg^{-1}) were at high levels. Conversely, low P application (20 mg kg^{-1}) and low microbial community (AS) levels were associated with the lowest AP_{ASE} activity (Table 4).

The P_{MIC} was shown to be highest with NS treatment and lowest under AS treatment, regardless of the P application. Similar results were found for different microbial communities, where the highest P_{MIC} was observed under no P application and the lowest P_{MIC} was observed under 20 mg kg^{-1} (Table 4).

For P_{UPT}, disregarding the P application, the highest value was observed under AS treatment. Moreover, the highest P doses (60 mg kg^{-1}) were found to produce the highest P_{UPT}, which was then followed by 40 and 20 mg kg^{-1}, and no P application produced the lowest P_{UPT} (Table 4).

2.3. Experiment 2: Mycorrhiza Effect on Shoot P Uptake of Plant Cultivation under Microbial Manipulation and P Fertilizer Rate Application

Overall, the inoculation of mycorrhiza spores (WMI) did not increase the mycorrhizal colonization (MYC) (Figure 2), presenting similar results to the non-mycorrhizal spore inoculation (NMI). However, the conditions created by the methods to distinguish the microbial community and the P application modulated the mycorrhizal colonization (MYC) and the P uptake (P_{UPT}) (Figure 2).

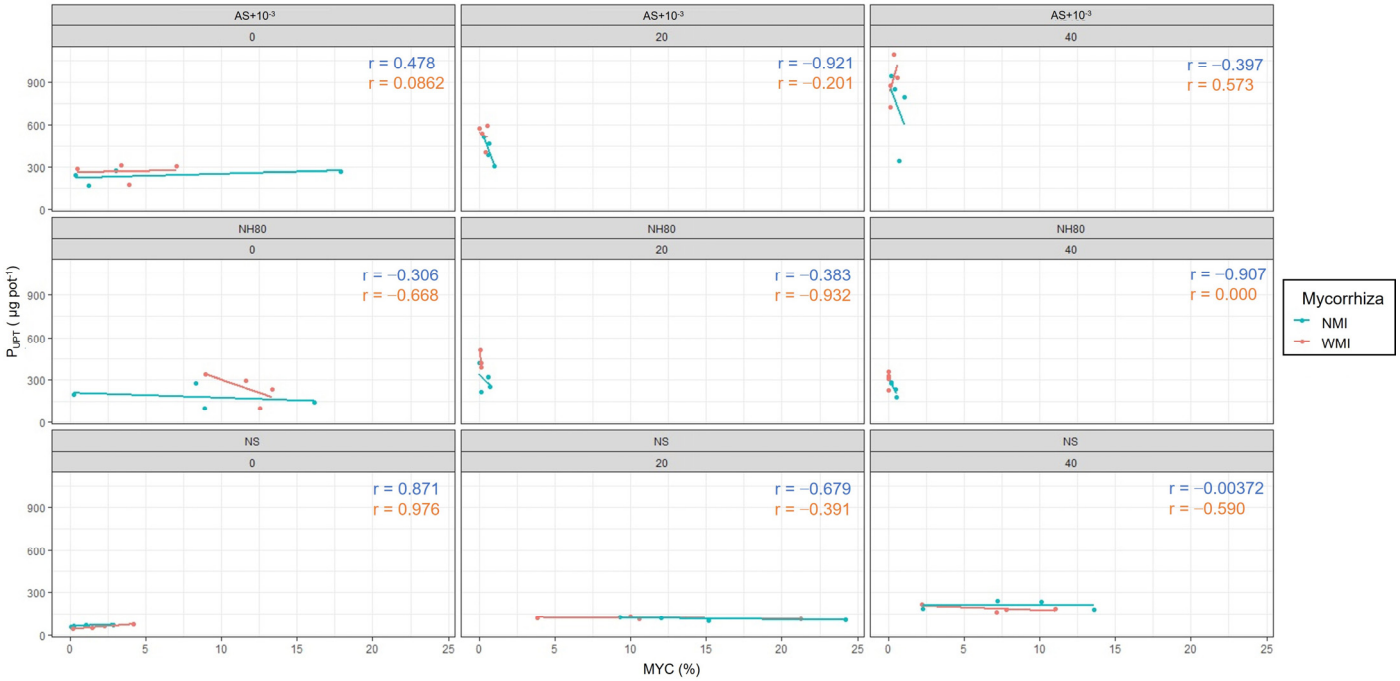

Figure 2. Relationship between P uptake (P_{UPT}) and mycorrhization rate (MYC) from samples taken after cropping with brachiaria in soils subjected to three soil microbial communities: three P rate application and mycorrhiza inoculation. Treatment abbreviations are as follows: autoclaved soil followed by re-inoculation with dilution from the natural soil (10% *w*/*v* of natural soil) (AS + 10^{-3}); soil heated at 80 °C for 1 h (NH80); and natural soil without manipulation (NS); P fertilizer was applied as triple superphosphate at the following rates: 0; 20; and 40 mg kg^{-1} of P; mycorrhiza inoculation: non-mycorrhiza inoculation (NMI) and with mycorrhiza inoculation (WMI).

High mycorrhization and P_{UPT} were observed under manipulated microbial communities (AS + 10^{-3} and NH80) and no P application (0 mg kg^{-1}), reaching a P_{UPT} around 300 µg kg^{-1}. This treatment showed the highest positive correlation values (r = 0.871 for NMI and r = 0.976 for WMI). In addition, the P_{UPT} that showed the highest values were obtained by applying intermediate and recommended P doses (20 and 40 mg kg^{-1}) and manipulating the microbial community (AS + 10^{-3} and NH80). However, in these treatments there was low mycorrhization (lower than 5%).

On the other hand, the natural soil condition (NS) under no P application showed low mycorrhization and low P uptake, but when brachiaria was cultivated under NS with an intermediate and recommended P dose application (20 and 40 mg kg^{-1}), they exhibited high mycorrhization values, reaching values of 5 to 25% of colonization, and a P_{UPT} about 300 µg kg^{-1} (Figure 2).

3. Discussion

3.1. Experiment 1: Manipulation of Soil Microbiome and P Dose Application

The autoclaving procedure itself reduced P availability and increased the concentrations of Fe and Mn. In the same way, Wolf et al. [15] and Razavi Darbar [16] also found that

autoclaving varied chemical properties such as soil pH, P, Mn, and Fe. These results may be due to the high temperature (121 °C) and pressure applied to the soil samples during the procedure, which beyond the microbial changes may promote chemical changes in the soil. However, most of the prior studies that examined soil sterilization procedures using autoclaving were published one or two decades ago and further studies are required.

On the other hand, to evaluate the biological impact of the methods applied, AP_{ASE} activity was used, as this is an enzyme related to the P cycling, promoting the mineralization of the organic P forms. Despite the low activity, the autoclaving and heating methods did not extinguish the soil AP_{ASE} activity. This enzyme may be released by plants and microorganisms and can be present freely in the environment in large quantities. Therefore, the results found in this study regarding AP_{ASE} may reflect both the reduction in the AP_{ASE} activity itself and the alteration in the microorganisms responsible for this enzyme's production and therefore directly related to the soil microbial activity. The reduction in the AP_{ASE} activity in the autoclaved soil (AS) may be linked to the decrease in the microbial activity and degradation of the enzymes by temperature and pressure, while the re-inoculation of microorganisms (AS + 10^{-3}) increased the AP_{ASE} activity in the autoclaved soil. The heating treatment, under atmospheric pressure and a temperature of 80 °C per 1 h, did not result in a significant reduction in the AP_{ASE} activity in comparison to that in natural soil (NS), indicating that both free form enzyme and microbial activity were affected, but minimally. Under natural conditions (NS), the AP_{ASE} activity showed the highest values, indicating initial enzyme activity, under an undisturbed condition, and P application did not affect its activity. As the AP_{ASE} activity is strongly correlated to the organic P forms, the addition of inorganic P may not alter its activity.

Regarding the abundance, an equalization among the treatments was expected after a period left for cell multiplication, where the treatments should present diversity differences, but abundance equality. However, an increase in the fungal abundance was observed in the autoclaved soil (AS and AS + 10^{-3}).

3.2. Experiment 1: P Dynamics after Plant Cultivation under Microbial Manipulation and P Fertilizer Rate Application

The multivariate analysis showed that brachiaria presented higher P immobilization in the microbial biomass, acid phosphatase activity, and P accumulated in the shoot, compared to crotalaria. This might be due to the fact that brachiaria is a grass with a fasciculate root system, which may help to explore high soil volumes and the exudates may enhance microbial activity [17].

Our results showed that brachiaria cultivated in undisturbed microbial communities, natural soil (NS), but no P application, lead to a reduction in the soil's available P levels while maintaining high acid phosphatase activity (AP_{ASE}) and high amounts of microbial P immobilization (P_{MIC}). Hence, under these conditions, the shoot P uptake was the lowest observed. Possibly, in a high-diversity condition, found in natural soil (NS), some microorganisms may use P more efficiently than the plants, resulting in a greater amount of P being immobilized in the microbial biomass, thus causing a temporary reduction in the availability of P to plants [18]. McLaughlin and Alston [19] observed a decrease in dry weight and P uptake of wheat (*Triticum* sp.) in the presence of ^{33}P-labelled residues of medic (*Medicago trunculata* cv. Paraggio) and concluded that this decrease was due to competition between the plants and soil microbial biomass for available P. Additionally, Turner et al. [20] observed that in mature ecosystems, soil P in the microbe biomass accounted for most of the total P compared to plant P. This emphasizes the central role of P_{MIC} in regulating P availability, as there is strong competition between plants and saprotrophic microbes for soil P. However, it is important to remark that microbes may potentially modulate P accumulation by efficiently cycling in comparison to the plants, which may be a strategy to avoid P adsorption in the soil surfaces and keep it available in the soil system for longer during plant development. Once the microorganisms die, the P may become available to the plants again, while the P adsorbed in the soil surface may not.

An opposing tendency was seen when brachiaria was grown on soils with limited diversity (AS and AS + 10^{-3}), but with high doses of P applied. In this case, low AP_{ASE} and P_{MIC} were observed, although there was a substantial P uptake. Enhancing the chemical availability of the nutrients may be compensated for other deficient factors, such as soil microbial community for plant P uptake. Therefore, the use of fertilizer may be an important strategy for chemical soil fertility restoration and minimizing low nutrient degradation risks [21]. Furthermore, a reduction in the competition between the microorganisms and the plants may be observed. In this way, Wertz et al. [22] raised the paradigm that soil microbial communities, due to high diversity, have high functional redundancy levels, and hence, the erosion of microbial diversity is less important for common ecosystem microbial functions, such as decomposition and respiration. These authors demonstrate that the vast diversity of the soil microbiota makes soil ecosystem functioning largely insensitive to biodiversity erosion, even for functions performed by specialized groups. Depending on the microorganisms extinguished from the system because of manipulation or degradation, the competition between the plants and the surviving microorganisms may be reduced, resulting in more P available for plant uptake. In this sense, Li et al. [23] observed higher growth of peach (*Prunus persica*), corn (*Zea mays* L.), and tomato (*Lycopersicon esculentum*) in sterilized soil compared to that in natural soil, and after cropping, the authors observed the presence of plant growth-promoting rhizobacteria in the rhizosphere, indicating a selection of beneficial organisms after sterilization. Li et al. [23] demonstrated that the rhizosphere community of a non-native plant cultivated in a sterile soil can be rapidly regenerated with the influence of the plant, and the new microbial community that develops is a healthier rhizosphere microbiome. Also, this sterilization of the soil led to a reduction in pathogens, which was previously observed by Sosnowski et al. [24]. Franklin and Mills [25], using the dilution-induced reduction in the diversity in sewage, observed that the original community was similar to the regrown community from an inoculum (10^{-6} dilution) and concluded that for each organism type eliminated during the dilution process, at least one species remained in the system to maintain its functionality, reducing the competition between species, according to functional redundancy [26].

Another factor to be considered is the brachiaria traits. Gorgone-Barbosa et al. [27] described *U. brizantha* as a fire-resistant species, revealing that this crop survived even after a fire event in the Brazilian savannah (Cerrado). This characteristic may favor brachiaria growth in an environment submitted to a stress condition, such as in autoclaved and heated soil experiments. This evidence might be based on how plants alter the rhizosphere, which in turn affects the microbiome vicinity and P availability, modifying the conditions to meet the nutrient demands [28–31].

Regarding crotalaria results, this crop maintained more labile P in the soil under high microbial community levels (NS), even without P application. Additionally, this crop was efficient in increasing P_{AER} in the manipulated soil (AS and NH80), even under no P input. This result indicates the strong potential of this crop to increase the P availability under unfavorable and disturbed chemically and biologically conditions. This effect is most likely attributed to the root system and exudates of this crop, favoring the maintenance of labile P in the soil even under no fertilization. This feature can serve as a strategic approach to keep soil P for the following crop, hence reducing the need for P application.

Similar to brachiaria, crotalaria increased AP_{ASE} under high microbial community and high P application. However, unlike brachiaria, crotalaria did not show any interaction between microbial communities and P application for P_{MIC} and P_{UPT}. Thus, there was no compensatory effect for these parameters. Apart from that, the trend between crotalaria and brachiaria was the same, where the lack of P application favored P immobilization but harmed plant P absorption regardless of the microbial community. Despite a microbial alteration, some beneficial microorganisms for plant P uptake may have persisted in the soil, even after soil manipulation, rendering the manipulation harmless to the plant P uptake.

Therefore, both brachiaria and crotalaria results showed that microorganisms can compete with plants for available P from the soil solution. This was observed by a significant

pool of immobilized P, making the P temporarily unavailable to plants. However, over the longer term, all microbial P may be potentially available to plants in a turnover process, suggesting that immobilization of P within the biomass can be considered an important mechanism for regulating the supply of P in the soil solution [32] and protecting the labile forms from adsorption processes in the soil particles [33].

3.3. Experiment 2: Mycorrhiza Effect on Shoot P Uptake of Plant Cultivation under Microbial Manipulation and P Fertilizer Rate Application

The methods used to reduce the microbial community (AS + 10^{-3} and NH80) under no P application promoted both mycorrhizal colonization (MYC) and P uptake by brachiaria. This may indicate that these methods, i.e., autoclaving and heating, did not eliminate the viability of the mycorrhizal spores of the soil, as mycorrhization was observed under both treatments, without and with mycorrhiza inoculation. Additionally, the increased Mn and Fe promoted by the autoclaving did not show to be a limiting factor for mycorrhization. Nogueira and Cardoso [34] reported that the colonization was not limited to the soil with up to Mn 300 mg dm^{-3} in soybean. In our study, after autoclaving, Mn reached a maximum of 34 mg dm^{-3}, which was unlikely to result in a reduction in mycorrhizal colonization. Furthermore, the autoclaving procedure promoted a reduction of P_{AER}. Altogether, the P_{AER} reduction promoted by the autoclaving and no P input treatment (0 mg kg^{-1}) may assist the establishment of the AM-symbiosis. Some authors report that low P availability stimulates the association between plant and mycorrhizal as a strategy to reach P in the soil. Another factor to be considered is the reduction in soil microbial community, generated in the manipulated conditions (AS + 10^{-3} and NH80), resulting in lower competition between plants and the soil microbiota of the available P in the soil, which benefits brachiaria, at this stage, for P uptake. However, these are conditions under low chemical and biological fertility, which is not sustainable in the long term, regarding general nutrient cycling and plant nutrition. Furthermore, although higher P uptake was found in low-diverse treatments, the roles of the soil microbiome in supplying other plant needs during their development cannot be neglected. Singh et al. [35] emphasized the effect on the loss in specific functions in environmental disturbance, such as by the presence of heavy metal, where some key specialized functions (carried out by specific groups) may be compromised.

On the other hand, the addition of mineral fertilizer in the manipulated systems (AS + 10^{-3} and NH80) inhibited the mycorrhization, as the symbiosis in this case may represent an unnecessary use of energy for the plants, as the mineral P input was efficient in improving the P_{UPT}. Therefore, under this condition, low competition situation is generated to the plant due to the reduction in the microbial community in AS + 10^{-3} and NH80, and the mineral P input guarantees the available P to the plants. However, this situation leads to a high chemical dependency on mineral P inputs.

Under natural condition (NS) and no P input (0 mg kg^{-1}), the colonization was observed in low rates and no significant increase in the P_{UPT} was observed. Some authors indicate that the soil microbial community may have negative, positive, or neutral effects on the mycorrhization. Also, this result may indicate that the competition between the microbial community and the plants for the available P negatively affects the P uptake. However, this condition showed a high positive correlation between mycorrhization and P_{UPT}, indicating that under low P and undisturbed microbial community, the mycorrhiza colonization is activated and helps to improve the P_{UPT}.

Conversely, under NS and P input treatments of 20 and 40 mg kg^{-1}, both the mycorrhization and P_{UPT} were beneficiated. This may be related to the minimal amount of P required for the symbiosis between plant roots and mycorrhiza fungi, as the P application corresponded to 50 and 100% of the recommend doses for brachiaria, which may stimulate the spores presented in the natural conditions (NS). Despite the presence of a higher microbial community reflected in lower P_{UPT} in comparison to conditions under lower microbial conditions and the same fertilization, the plant presents a balance with mycorrhization and P_{UPT}.

Analyzing the compensatory effect between chemical and biological fertility, the increase in P input (20 and 40 mg kg^{-1}) under low microbial condition (AS + 10^{-3} and NH80) allowed an increase in the P uptake but not the mycorrhizal symbiose. This indicates that the focus on the chemical fertility improves the P acquisition by the plants but harmed the relationship between the agents in the soil. On the other hand, under no P input, in a soil with low available P, such as tropical and subtropical soils, the original microbial community (NS), without any interference to promote its reduction, showed low mycorrhizal symbiosis rates and low P_{UPT}. This indicates that to maintain the microbial community is not enough to promote P acquisition to the plants or symbiosis under low availability of P. Finally, the undisturbed microbial community (NS) and the P input, in the recommended doses to brachiaria, allowed the symbiotic relationship between the brachiaria and mycorrhiza, with satisfactory P_{UPT}, indicating that the best scenario is the balance between the chemical and biological status of an agroecosystem.

3.4. Limitations and Outlooks

The methods used here (autoclaving and heating) were selected due to their accessibility and low cost in most of the laboratory facilities, particularly when considering the reproducibility factor for further experiments. In addition, these methods are well known amongst soil scientists to reduce the soil microbial community in their indoor experiments as organisms sensitive to high temperature are eliminated from the system. Furthermore, autoclave and ovens are commonly found in laboratory facilities and are cost-effective. However, the methods used to distinguish the microbial community had an impact on P_{AER}, which is the focus of this study. Despite the changes promoted by the used methods on P_{AER}, crotalaria was able to promote a change in the available P_{AER} in the autoclaved soil. This is an important finding regarding the biological potential to induce changes and improve the chemical and biological soil fertility, regardless of the initial P_{AER} soil content. To prevent chemical changes, mainly regarding P_{AER}, other methods that aimed to sterilize the soil, which not only rely on temperature as a principle, may be used. In this sense, some sterilization methods have been described to prevent changes in the parameter such as P, including the use of gamma ray. However, this technique limits the sterilized soil amount over time and is much more expensive.

Another limitation factor to be considered is the assessment of the soil microbial community reduction caused by the autoclave procedure, particularly when using methods based on DNA analyses, such as quantitative polymerase chain reactions (qPCR). This method may overestimate microbial diversity in the autoclaved soil (AS) as it may lead to an absence of microbial activity in the soil after the procedure, while the death DNA remains in the soil as relic DNA [36]. In this sense, the presence of relic DNA in the autoclaved soil may present a similarity in diversity when compared to the non-autoclaved soil (in our study, natural soil—NS), leading to an apparent similarity when it is nonfunctional, as reported by Arruda et al. [37]. Once the autoclaved soil is re-inoculated with microorganisms, in our study represented by AS + 10^{-3} treatment, the relic DNA might be consumed by the reinserted microbiota, thus reducing the overestimation.

Despite the limitations, the results obtained in this study raise the outlooks for the importance of the balance between chemical and biological fertility in an agroecosystem, as the reduction of either chemical or biological fertility will affect the soil quality.

4. Materials and Methods

Two greenhouse (27 ± 3 °C) experiments were conducted as described in the following sections. For both experiments, each experimental unit comprised a plastic pot (5 cm internal diameter × 9 cm tall) containing 270 g of soil (dry weight). Soil was classified as a sandy clay loam textured Ferralsol [38], collected from a depth of 0–20 cm within a livestock-grazed pasture, in Piracicaba, São Paulo, Brazil (22°43′03.0″ S; 47°37′00.4″ W). The soil used in both experiments showed the chemical fertility, determined by using the methodologies proposed by Raij et al. [39], as follows: soil organic matter 38 g dm^{-3};

S 11 mg dm^{-3}; K 0.9 $mmol_c$ dm^{-3}; Ca 16 $mmol_c$ dm^{-3}; Mg 7 $mmol_c$ dm^{-3}; B <0.15 mg dm^{-3}; Cu 0.8 mg dm^{-3}; Fe 77 mg dm^{-3}; Mn 5.8 mg dm^{-3}; Zn 3.2 mg dm^{-3}; pH_{CaCl2} 5.0; Al 1.0 $mmol_c$ dm^{-3}; H + Al 25 $mmol_c$ dm^{-3}.

4.1. Experiment 1: Manipulation of Soil Microbiome and P Dose Application

The initial step of the first experiment was stablished in a factorial design (4 × 4), with eight replicates, totaling 128 experimental units. Four different soil microbial communities were selected based on a previous study [37] that used eight soil microbial communities: (i) natural soil (NS); (ii) soil exposed to heat treatments for 1 h at 50 °C, (iii) 80 °C, or (iv) 100 °C; sterilized soil by autoclaving (AS) followed by re-inoculation of dilutions of the natural soil community at (v) AS + 10^{-1}, (vi) AS + 10^{-3}, and (vii) AS + 10^{-6}; and (viii) AS without re-inoculation. The selection of the treatments for the present study was based on the extreme treatments for the lowest and the highest microbial communities, and for each method, intermediate treatments were selected. Therefore, the soil microbial communities used the following methods: (i) sterilizing the soil by autoclaving twice (121 °C, 103 kPa, 1 h) at a 24 h interval, used as a negative control; (ii) after 7 d, the autoclaved soil was re-inoculated with a dilution (10% *w*/*v* of unmanipulated soil), based on the dilution-to-extinction gradient approach [40] (AS + 10^{-3}); (iii) a heat method [41] was applied by heating the natural soil at 80 °C for 1 h (NH80); and (iv) the natural soil (NS) without manipulation, used as a positive control. After the microbiome was established, P fertilizer triple superphosphate (TSP; 45% soluble P_2O_5) was applied at the following rates: 0, 20, 40, and 60 mg kg^{-1}, corresponding to approximately 0, 40, 80, and 120 kg ha^{-1} of P_2O_5. The doses were based on 0, 50, 100, and 150% of the farmer's recommendation for brachiaria and crotalaria cultivation, according to the manual of recommendations to São Paulo state, Brazil [42].

After setting up the microbial and P treatments, soil moisture was kept at 70% of the maximum holding capacity (60 mL pot^{-1}), using sterilized deionized water to promote cell multiplication of the differentiated communities and the re-establishment of the microbial community abundance. After 20 d, the soil was sampled for chemical and biological soil characterization.

For chemical characterization, soil samples were taken from the treatments (3 × 4): AS, NH80, and NS; and 0, 20, 40, and 60 mg kg^{-1} of P input. The soil samples were air-dried and analyses of available macro and micronutrients were performed [39]: soil organic matter (SOM); macronutrients (S; K; Ca; Mg; P_{AER}); micronutrients (B; Cu; Fe; Mn; Zn); and acidity attributes (pH_{CaCl2}; Al; H + Al).

For biological characterization, soil samples from the treatments (4 × 4), AS, AS + 10^{-3}; NH80, and NS, and 0, 20, 40 and 60 mg kg^{-1} of P input, with four replicates, were taken for the determination of the acid phosphatase activity (AP_{ASE}; mg kg^{-1} h^{-1}) (soil sample was kept in 4 °C until analysis) and bacterial and fungal quantification (soil sample was kept frozen in –20 °C until analysis). Acid phosphatase activity (AP_{ASE}) analysis was performed according to Tabatabai and Bremner [43]. From the frozen soil samples, DNA was extracted using the PowerSoil DNA isolation kit (MoBio, Carlsbad, CA, USA), according to the manufacturer instructions. Quality of the extracted DNA was assessed in a 1% (*w*/*v*) agarose gel, followed by staining with ethidium bromide and photo documentation of ultra-violet light (transluminator, Storm 845—GE Healthcare Life Sciences, Piscataway, NJ, USA). The quantitative polymerase chain reaction (qPCR) of the V4 region of the bacterial 16S rDNA gene amplification was performed with the set of primers P1 (Eub338) and P2 (Eub518) [44]. The qPCR reactions were performed using SYBR® green-based quantification in the StepOne™ Real-Time PCR System (Applied Biosystems, Darmstadt, Germany). For the reaction, an initial denaturation step at 95 °C for 10 min; 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s; and a final extension step at 72 °C for 7 min were performed to confirm that all the treatments matched a similar microbial abundance. The qPCR of the internal-transcribed spacer (ITS) amplification was performed with the set of primers ITS1f and 5.8s [45]. The qPCR reactions were performed using SYBR® green-based

quantification in the StepOne™ Real-Time PCR System (Applied Biosystems, Germany). For the reaction, an initial denaturation step at 94 °C for 15 min; 40 cycles of 94 °C for 60 s, 53 °C for 30 s, and 72 °C for 60 s; and a final extension step at 72 °C for 10 min were performed to confirm that all the treatments matched a similar microbial abundance. The 16S reactions were run in 20 µL comprising 10 µL of SYBR® Green, while ITS reactions were run in 25 µL comprising 12.5 µL of SYBR® Green. All qPCRs received 0.2 µL (0.2 µM) of each primer, 1 µL of DNA template, and nuclease-free sterile water.

For the chemical features, a descriptive analysis was performed for the nutrients that presented a minimal difference in the values among the treatments, using a red–green color scale to represent the results for the variable individually, considering the factors' combination: quantitative (levels of phosphate application) and qualitative (level of microbial communities), where red was attributed to the lowest value and green to the highest value. For biological parameters (AP_{ASE}, bacteria qPCR, and fungal qPCR), the gamma regression model was used and the following models: (i) null model (intercept only), (ii) without interaction, and iii) with interaction. The Akaike Information Criterion (AIC) was used to select the most suitable model, by using Student's *t*-test ($p \leq 0.05$). For each data set, a red–green color scale was used to represent the results for the variable individually, considering the factors' combination: quantitative (levels of phosphate application) and qualitative (level of microbial communities), where red was attributed to the lowest value and green to the highest value.

4.2. Experiment 1: P Dynamics after Plant Cultivation under Microbial Manipulation and P Fertilizer Rate Application

For the cultivation stage of the first experiment, seeds of brachiaria (*Urochloa brizantha* cv. Marandu) and crotalaria (*Crotalaria juncea* L.) were used. For the implementation, the seeds were disinfected with 2% (*v*/*v*) NaClO solution (15 min) and rinsed in sterilized deionized water. Germination was carried out in sterile and P-free sand. The seedlings were watered with sterilized deionized water until they were transplanted into the pots with the treatments in a factorial design 4 × 4. The first factor was the microbial community manipulations: AS, AS + 10^{-3}, NH80, and NS, and the second factor was the P rates: 0, 20, 40, and 60 mg kg^{-1}, using 4 replicates. Therefore, a total of 64 pots were utilized for each plant, i.e., brachiaria and crotalaria. The transplant of seedlings (brachiaria: 5 seedling pot^{-1}; crotalaria: 1 seedling pot^{-1}) occurred 20 d after the manipulation of the soil microbiome and P application. The pre-seedlings (5 d old) were immersed in sterilized deionized water (30 min) to facilitate their removal from the sand. Pots with the newly transplanted seedlings remained in a room for 48 h with indirect light. Afterwards, they were taken to the greenhouse, where they remained for 28 d, until the experimental harvest. During this period, the pots were irrigated with sterilized deionized water to maintain 70% of the maximum water-holding capacity (60 mL pot^{-1}).

After 30 d of cultivation, the plants were removed from the pots for soil and shoot sampling. A portion of the soil was air-dried to analyze the inorganic labile P fraction extracted with anion exchange resin (P_{AER}; mg kg^{-1}) [46] and determined by colorimetric assay [47]. Another portion of the soil was stored in a cold room (4 °C) for acid phosphatase activity enzyme (AP_{ASE}; mg kg^{-1} h^{-1}) analysis [43] and microbial P biomass (P_{MIC}; mg kg^{-1}) determinations [48]. The plant shoot was dried in an oven (55 °C) until stabilization of the weight to obtain the shoot dry matter (SDM; mg pot^{-1}). After, the dried shoot was individually triturated using liquid nitrogen with a ceramic crucible and pistil. The resulting powder was then sieved through 150 µm mesh, and 30 mg of it was used for the P analysis content (P_{CON}; µg mg^{-1}). P_{CON} was determined using the energy-dispersive X-ray spectroscopy technique. P uptake (P_{UPT}; µg pot^{-1}) was calculated by using Equation (1):

$$P_{UPT} = SDM \times P_{CON} \quad (1)$$

where SDM is the shoot dry matter and P_{CON} is the P content in the shoot.

For statistical analyses, principal component analysis (PCA) was used to evaluate the relationships among P_{AER}, AP_{ASE}, P_{MIC}, and P_{UPT} and the trend in relation to the crops. To evaluate the treatments, microbial communities (AS, AS + 10^{-3}, NH80, and NS), and phosphate application (0, 20, 40, and 60 mg kg^{-1}) in relation to each of the variables ($P_{AER,}$ $AP_{ASE,}$ P_{MIC}, and P_{UPT}), the gamma regression model was used and the following models: (i) null model (intercept only), (ii) without interaction, and (iii) with interaction. The Akaike Information Criterion (AIC) was used to select the most suitable model, by using Student's *t*-test ($p \leq 0.05$). The red–green color scale was used to represent the results considering all the data sets for each variable, with the factors' combination: quantitative (levels of phosphate application) and qualitative (level of microbial communities), where red was attributed to the lowest value and green to the highest value.

4.3. Experiment 2: Mycorrhiza Effect on Shoot P Uptake of Plant Cultivation under Microbial Manipulation and P Fertilizer Rate Application

Based on the results obtained in the first experiment, this second experiment was performed in a factorial design (3 × 3) with four replicates, totaling 36 experimental units. Three different soil microbial communities were used: (i) the autoclaved soil, sterilized by autoclaving twice (121 °C, 103 kPa, 1 h) at a 24 h interval, re-inoculated (after 7 d after sterilization) with a dilution (10% w/v of unmanipulated soil, based on the dilution-to-extinction gradient approach [40]) (AS + 10^{-3}); (ii) a heat method [41] was applied by heating the natural soil at 80 °C for 1 h (NH80); and (iii) the natural soil (NS) without manipulation, used as a positive control. After the microbiome was established, P fertilizer triple superphosphate (TSP; 45% soluble P_2O_5) was applied at the following rates: 0, 20, and 40 mg kg^{-1} of P, corresponding to approximately 0, 40, and 80 kg ha^{-1} of P_2O_5. The doses were based on 0, 50, and 100% of the farmer's recommendation for brachiaria cultivation, according to the manual of recommendations to São Paulo state, Brazil [42].

For the cropping stage, seedlings of brachiaria were transplanted (5 seedlings pot^{-1}) 20 d after the manipulation of soil microbiome and P application treatments. The pre-seedlings (5 d old) were immersed in sterilized deionized water (30 min) to facilitate their removal from the sand. During the transplanting, two different mycorrhiza treatments were carried out: (i) without mycorrhiza inoculation (NMI); and (ii) with mycorrhizal inoculation (WMI). For the inoculation, a solution containing spores of mycorrhiza (*Rhizophagus clarus*) were inoculated directly into the roots (10 mL of solution to each set of roots per pot). The spore solution contained ca. 18 spores mL^{-1} in 500 mL sterile deionized water and was maintained at 4 °C until inoculation. The inoculum was acquired from the collection of the Department of Soil Science/Soil Microbiology Laboratory, "Luiz de Queiroz" College of Agriculture, University of São Paulo. Pots with the newly transplanted seedlings remained in a room for 48 h with indirect light. Afterwards, they were taken to the greenhouse, where they remained for 28 d, until the experimental harvest. During this period, the pots were irrigated with sterilized deionized water to maintain 70% of the maximum soil water-holding capacity (60 mL pot^{-1}).

After 30 d of cultivation, the plants were removed from the pots for shoot and root sampling. Plant shoot was dried in an oven (55 °C) until stabilization of the weight to obtain the shoot dry matter (SDM; mg pot^{-1}). After, the dried shoot was individually triturated in liquid nitrogen with a ceramic crucible and pistil, later sieved through 150 µm mesh, and 30 mg was used for the P content analysis (P_{CON}). P_{CON} was determined using the energy-dispersive X-ray spectroscopy technique. P uptake (P_{UPT}; µg pot^{-1}) was calculated by using Equation (1). Plant root samples were kept in 70% ethanol until processing in the laboratory. First, the roots were bleached (KOH 10%; 90 °C; ~20 min) in a water bath. Then, the roots were dyed (blue ink, 90 °C; ~5 min). For each root sample, three slides were made containing ten root segments (~1 cm length) per slide. Thus, in total, 30 segments were analyzed per sample. Mycorrhizal root colonization rates were assigned according to the number of fungal structures in the entire 1 cm length, as described by Trouvelot

et al. [49], varying from 0%, where no colonization was observed, to > 90%, for segments fully colonized by the arbuscular mycorrhizal fungi.

For statistical analyses, data of P uptake (P_{UPT}) and mycorrhization (MYC) were plotted using a scatterplot for each factorial combination (3 × 3) (AS + 10^{-3}; NH80; and NS) and (0; 20; and 40 mg kg^{-1} of P). The straight-line adjustment was performed by simple linear regression, and Pearson's correlation coefficients were obtained for the treatments: non mycorrhiza inoculation (NMI) (n = 4) and with mycorrhiza inoculation (WMI) (n = 4).

5. Conclusions

Plant species greatly influenced the compensatory effect of P application and microbial community manipulation on plant P uptake. Brachiaria, a grass, had the greatest compensating effect, where the increase of P in the soil caused by phosphate application in the presence of low microbial community improved the plant capacity to uptake P from the soil. However, under low P input, the increase in the soil biodiversity was insufficient in improving shoot P uptake. Crotalaria showed no compensating effect, but showed a higher P uptake under high P application regardless of the microbial diversity. Likewise, the same pattern was observed under a low microbial community regardless of the P application. In both cases, the reduction in the microbial community evidenced a temporary decrease in microbial competition between microorganisms and plants, which promoted higher P shoot accumulation.

In this direction, considering brachiaria mycorrhization and P_{UPT} data, an increase in the P_{UPT} under low microbial communities (AS + 10^{-3}; NH80) with P addition (20 and 40 mg kg^{-1} of P) was observed, while no mycorrhization was established. Meanwhile, under an undisturbed microbial community (NS), under no P input (0 mg kg^{-1} of P), low mycorrhization and low P_{UPT} were observed. Finally, NS and the recommended P input (40 mg kg^{-1} of P) represented a balance between chemical and biological fertility, promoting the equilibrium between mycorrhization and P_{UPT}.

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