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Received: 15 February 2019; Accepted: 8 April 2019; Published: 15 April 2019

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Abstract: To maintain grassland productivity and limit resource depletion, scarce mineral P (phosphorus) fertilizers must be replaced by alternative P sources. The effect of these amendments on plant growth may depend on physicochemical soil parameters, in particular pH. The objective of this study was to investigate the effect of soil pH on biomass production, P use efficiency, and soil P forms after P amendment application (100 mg kg^{-1} P) using poultry manure compost (PM), rock phosphate (RP), and their combination (PMRP). We performed a growth chamber experiment with ryegrass plants (*Lolium perenne*) grown on two soil types with contrasting pH under controlled conditions for 7 weeks. Chemical P fractions, biomass production, and P concentrations were measured to calculate plant uptake and P use efficiency. We found a strong synergistic effect on the available soil P, while antagonistic effects were observed for ryegrass production and P uptake. We conclude that although the combination of PM and RP has positive effects in terms of soil P availability, the combined effects of the mixture must be taken into account and further evaluated for different soil types and grassland plants to maximize synergistic effects and to minimize antagonistic ones.

Keywords: Poultry manure; phosphate rock; ryegrass; plant biomass; phosphorus uptake; phosphorus availability

1. Introduction

Fertilization of grasslands with mineral phosphorus (P) fertilizer is a common practice in many regions of the world to maintain productivity, especially on soils with high P retention [1–3]. In order to reduce the use of scarce phosphate rock (RP), alternative P fertilizers need to be found [4,5]. In this context, poultry manure, an abundant organic waste material from the growing broiler industry [6], is known for its high P content [7]. Its transformation through composting into organic amendments, and their subsequent application in grassland systems may be a promising strategy [8,9] to reduce the use of mineral fertilizers. Several studies showed that plant nutrient uptake and the biomass of several plants could be significantly increased using poultry manure compost (PM) [10,11]. The application of PM led to changes in soil P forms and phosphatase activity [12]. However, despite its positive effects on plant nutrient availability and biomass production, the application of PM may lead to the simultaneous introduction of contaminants [13] and could also lead to a loss of P to waterways following long term application.

Therefore, nowadays, the use of PM in combination with RP has been considered as good practice to limit the use of both materials without compromising plant requirements [14]. However, the fertilizer value of both substrates may depend on the soil reaction. For example, RP efficiency may be limited in soils with high pH due to its low dissolution rate [15], while in soils with acid pH, RP may lead to further acidification [16]. The efficiency of PMRP mixture for increasing wheat and chili yield and P uptake has already been demonstrated for acid and alkaline soils [17,18]. However, no study has been carried out to investigate quantitatively the synergetic or antagonistic effects of the combined application of both materials.

In this study, we carried out a growth chamber experiment to investigate the effect of the combined use of PM and RP as compared to their application as a single amendment in soils with similar properties, but contrasting in pH. The objective of the study was to determine the effect of PM application, alone or combined with RP, on ryegrass biomass production, P use efficiency, and soil P availability in an acid and an alkaline soil. We hypothesized that the soils' and plants' response to the combined use of PM and RP in terms of biomass production and P use efficiency may depend on the soil reaction and that the mixture will have additional effects as compared to the use of PM and RP as a single amendment. Moreover, we hypothesized that the combined use of PM and RP will ameliorate P availability and biomass production as compared to their use as a single amendment.

2. Materials and Methods

2.1. Materials

We used two silty soils (50–60% silt): A Neoluvisol with a pH of 6.1 (moderately acid soil) and a carbonated Luvisol, with a pH of 8.5 (alkaline soil) (Table 1) according to the French Référentiel Pédologique 2008 [19]. Both soils showed similar texture, organic matter content, and soil forming processes (lixiviation). They were differentiated by pH and also their initial Olsen P concentration. They are part of the French observatory SOERE PRO (https://www6.inra.fr/valor-pro/SOERE-PRO-les-sites). The Neoluvisol is located in Eastern France at Colmar, and the Luvisol is located in northwestern France in Brittany at Le Rheu. We sampled the first 0 to 30 cm. of the control plots without fertilization at the two sites. After sampling, the soils were transported to the laboratory, air dried, and sieved at 2 mm. The plant species used was ryegrass (*Lolium perenne*), a typical pasture plant used for grazing systems.

Table 1. Soil physical and chemical characterization.

Soil Type	pH	C_{org} g kg^{-1}	C/N	P olsen mg kg^{-1}	K_2O Cmol + kg^{-1}	Clay %	Silt	Sand
Moderately acid	6.1	11.9	10	60	0.32	14.6	68.3	16.1
Alkaline	8.5	12.1 *	10	11	0.26	20.7	59.8	6.8

* $CaCO_3$ = 128 g kg^{-1}.

PM compost was provided by KOMECO B.V in pellet form with a dry matter content of 880 g kg^{-1} with an organic matter content of 600 g kg^{-1}. Phosphorus content was 13.2 g kg^{-1} d.w. The material contained 42% organic P and 58% of inorganic P (Table 2). RP was bought from 'Les comptoirs de Jardin' and was derived from bones with 30% P and 50% calcium. It was provided in powder form with 90% of the particles smaller than 0.16 mm.

Table 2. Inorganic (Pi) and organic P (Po) in fractions sequentially extracted from poultry manure compost PM.

H_2O		$NaHCO_3$		NaOH P mg kg^{-1}		HCl		Residual
Pi	Po	Pi	Po	Pi	Po	Pi	Po	Pt
187 ± 8	122 ± 43	202 ± 3	149 ± 30	47 ± 4	72 ± 13	181 ± 9	97 ± 8	145 ± 7

2.2. Growth Chamber Experiment

The experiment was carried out in pots in the RUBIC V biogeochemical reactor—Servathin, Carrières-sous-Poissy France—for 7 weeks. To account for the contrasting bulk densities, we amended 490 g of the moderately acid soil and 550 g of the alkaline soil per treatment, with four replicates with poultry manure compost (PM), phosphate rock (RP), or their mixture consisting of 70% PM and 30% RP (PMRP). In total, 100 mg P per kg^{-1} soil d.w. were added to each treatment. The amendments were supplied in the form of a dry powder. We added 13.30 g of PM and 0.80 g RP to the pots with a single amendment and 9.31 g of PM and 0.23 g of RP to the pots with amendment mixtures. To account for N and K supplied by PM (262 mg N and 221 mg K per kg d.w. soil), we added the corresponding amounts of K and N in the form of KCl and NH_4NO_3 to all other treatments, including the control. The PM application was equivalent to 9.8 Mg ha^{-1} when applied in the mixture with RP and 14 Mg ha^{-1} when applied as a single amendment. The RP application was equivalent to 0.25 Mg ha^{-1} when applied in a mixture with PM and 0.8 Mg ha^{-1} when applied as a single amendment.

After addition of the amendments, the soils were thoroughly mixed in plastic bags, added to each pot, and brought to field capacity with tap water. After one day, a total of 97 ryegrass seeds were added to each pot. Seeds were sown on the surface and covered superficially with soil material. Plants were grown at 24 °C (day temperature) and at 17 °C (night temperature) with a day length (light intensity of 650 μmol m^{-2} s^{-1}) of 8 h for the first 13 days, and 11 h until the end of the experiment. Soil moisture was maintained at 40% of the available field capacity by watering regularly. Air humidity was 75% to 65, % respectively, for day and night conditions.

After 7 weeks, shoots and roots were separated from soil and their fresh biomass was weighed. Thereafter, biomass was dried at 65 °C for 48 hours. Oven-dried plant material was ground to pass through 20-mesh (0.84 mm) sieves. Microbial biomass was determined using 5 g of fresh samples. The remaining soil masses were oven-dried at 40 °C and sieved at 2 mm. An aliquot was ground for further analyses. All data is expressed on a dry weight basis.

2.3. Soil Analysis

Phosphorus forms based on P solubility were measured using a modified Hedley fractionation scheme [20] with successive chemical P extraction from soluble to residual fractions. Briefly, 1 g of dry and sieved soil was extracted sequentially by shaking for 16 h with 30 mL of (1) distilled water, (2) 0.5 M $NaHCO_3$ at pH 8.5, (3) 0.1 M NaOH, and (4) 1 M HCl. Each suspension was centrifuged at 10,000 rpm for 10 min and the supernatants were recovered and analyzed for total P (Pt) and inorganic P (Pi). Organic P (Po) was determined by difference. The residues were dried at 60 °C and used for subsequent extractions. Residual P remaining after the last step was extracted with 1 M sulphuric acid (H_2SO_4) during 24 h, after calcination of the residue for 1 h at 550 °C. Inorganic P was determined in the solutions by the ammonium molybdate-ascorbic acid method [21]. Total P was determined by taking aliquots from supernatants for digestion using potassium persulfate ($K_2S_2O_8$) and 2.5 M sulphuric acid (H_2SO_4). Fraction one and two represent readily available P. Moderately available P is found in fraction 3, less available P in fraction 4, whereas residual P represents unavailable P. Pt, Po and Pi of bulk soil were calculated as sum of Hedley fractions.

Total organic C and N concentrations of soil samples were determined using an elemental analyzer (Variopyrocube, Elementar, Langensebold, Hesse, Germany). The acid soil was carbonate free and total soil C therefore corresponded to organic C. For the alkaline soil (carbonated soil), HCl-fumigation was performed before elemental analyses to remove inorganic C [22]. Microbial biomass P was determined by the chloroform fumigation-extraction method [23]. Briefly, 5 g of fresh soil were extracted with 0.5 M $NaHCO_3$ before and after fumigation with $CHCl_3$. Total P was determined in the solutions by the ammonium molybdate-ascorbic acid method [21]. Microbial biomass P was calculated as the difference between fumigated and non-fumigated soil and multiplied by a factor of 0.40 [23].

2.4. Biomass Analysis

Total N and C concentrations were measured using an elemental analyzer (Variopyrocube, Elementar, Langensebold, Hesse, Germany). Total shoot P contents were analyzed by calcination followed by acid recovery using inductive coupled plasma mass spectrometry (iCAPTM Q ICP-MS, Thermo ScientificTM, Waltham, MA, USA). The P uptake (mg) was calculated as a product of the shoots' or roots' nutrient concentrations (mg g^{-1}) and shoot or root biomass (g). Nutrient use efficiency (PUE) was also calculated according to Baligar et al. [24] as follows:

$$PUE = \frac{P\ uptake\ in\ treatment\ (mg) - P\ uptake\ in\ control(mg)}{total\ P\ applied} \times 100 \quad (1)$$

2.5. Synergistic and Antagonistic Effect of Mixture

Based on the quantities of PM and RP applied as single amendments or in mixture (see 2.2), we calculated the additional effect of the PMRP mixture on the soil available P, biomass production, and P uptake resulting from the combined application of PM and RP as compared to their use as a single amendment. This calculation is justified by the observation made by many others that PM would lead to enhanced mineralization of RP due to the release of organic acids (exp. 18). However, this additional effect was never quantified. We used Equation (2) to calculate the additional effect, i.e., change of PMRP as compared to the sum of single amendments:

$$\%\ change = \frac{observed\ result - expected\ result}{expected\ result} \times 100 \quad (2)$$

The observed result was corrected for the control in order to obtain the effect of the amendments. The expected result for PMRP was obtained as a sum of the PM and RM after multiplication of the observed results in the two treatments with a coefficient accounting for the different proportions of the two amendments used in the mixture, i.e., 70% and 30%.

2.6. Statistical Analysis

The experiment was arranged in a completely randomized design with four replicates. Data were checked for normality (Shapiro-Wilk test) and homogeneity of variance (Levene test) were determined before analyses. Statistical differences of means (95% significance level) were analyzed using two-way analyses of variance (two-way ANOVA). Post hoc tests with the function Tukey-test were made for the explanatory variables independently when the ANOVA detected significant differences. The relationships between soil available P and plant parameters were tested by Pearson correlation analyses. Statistical testing was done using the statistical program R Foundation for Statistical Computing Version 1.1.456 (R Development Core Team 2009–2018); effects were deemed significant at $p \leq 0.05$. Principal component analysis (PCA) was performed using the package, Factoextra; we consider one for the soil P fractions and a second one for the plant P uptake and biomass.

3. Results

3.1. Total Soil C, N, and P Concentrations

After the end of the experiment, PM treatment led to significantly increased total soil C concentrations in the moderately acid and alkaline soil by 57 and 29%, respectively (Table 3). The RP treatment showed no differences as compared to control, while its combination with PM increased the total C concentrations in both soils, with significant effects only in the moderately acid soil. Total N concentration was increased by 29% using PM in the alkaline soil. In the moderately acid soil, PM treatment increased total soil N concentration by 57%, whereas a lower increase was noted in the PMRP treatment (30%). Total P increased in both soils with PM amendment.

Table 3. Soil parameters in control and soil amended with poultry manure compost (PM), phosphate rock (RP), and their combination (PMRP) after 7 weeks of ryegrass growth.

Soil Type		C	N	C/N	Pt	C:Po	C:Pi	N:Pi	N:Po
		g kg^{-1}			mg kg^{-1}				
Moderate acid	Control	10.5 ^{Ca} *	1.2 ^{Da}	8.9 ^{Aa}	100.2 ^{Ca}	344.9 ^{Ba}	192.2 ^{Bb}	21.6 ^{Cb}	38.6 ^{Ba}
	RP	11.0 ^{Ca}	1.4 ^{Ca}	7.7 ^{Ba}	97.3 ^{Ca}	314.4 ^{Cb}	194.4 ^{Bb}	25.3 ^{Bb}	40.9 ^{Bb}
	PM	16.5 ^{Aa}	2.0 ^{Aa}	8.5 ^{Aa}	132.1 ^{Aa}	356.4 ^{Ba}	255.9 ^{Aa}	30.2 ^{Aa}	42.1 ^{Ba}
	PMRP	13.7 ^{Ba}	1.7 ^{Ba}	8.3 ^{Aa}	118.0 ^{Ba}	414.9 ^{Aa}	210.5 ^{Aa}	25.3 ^{Bb}	49.8 ^{Aa}
Alkaline	Control	11.5 ^{Ba}	1.3 ^{Ba}	9.2 ^{Aa}	103.2 ^{Ba}	365.3 ^{Ba}	241.9 ^{Aa}	26.3 ^{Ba}	39.8 ^{Ba}
	RP	12.0 ^{Ba}	1.7 ^{Ab}	7.2 ^{Aa}	103.5 ^{Ba}	399.7 ^{Aa}	239.2 ^{Aa}	33.3 ^{Aa}	55.6 ^{Aa}
	PM	14.8 ^{Aa}	1.7 ^{Ab}	8.8 ^{Ba}	127.2 ^{Aa}	383.2 ^{Ba}	244.1 ^{Aa}	28.1 ^{Ba}	44.3 ^{Ba}
	PMRP	13.4 ^{ABa}	1.6 ^{Aa}	8.7 ^{Aa}	120.8 ^{Aa}	275.0 ^{Cb}	290.1 ^{Aa}	33.5 ^{Aa}	31.8 ^{Bb}

* Upper case letters denote significant differences ($p \leq 0.05$) between treatments for one soil. Lower case letters denote significanct differences ($p \leq 0.05$) between soils for one treatment.

The C:N decreased in both soils amended with RP as compared with PM, PMRP, and the control (Table 3). In the moderately acid soil, PMRP treatments showed the highest N:Po and C:Po, while for the alkaline soil, the use of the mixture led to an increase of N:Pi, while C:Po and N:Po decreased. C:Pi was increased by both PM and PMRP treatments in the moderately acid soil only. N:Pi increased in all treatments in the moderately acid soil, while in the alkaline soil, only PMRP and RP increased this ratio significantly as compared with the control (Table 3).

3.2. Soil Phosphorus Forms

Concentrations of readily available fractions (extractable with water and $NaHCO_3$) ranged between 5.20 to 14.94 mg P kg^{-1} soil for inorganic and 6.07 to 27.58 mg P kg^{-1} soil for organic P (Figure 1). The moderately available fraction (NaOH extractable) ranged between 6.07 and 27.58 mg P kg^{-1} soil. Soils treated with PM and PMRP increased significantly inorganic and organic P concentrations in the readily available fraction as compared with the control and RP treatment. In the alkaline soil, RP amendment increased readily available inorganic and organic P on average 1.2-fold as compared with the control. No differences were found for the moderately acid soil (Figure 1). In the other fractions, the P concentrations ranged between 22.52 to 39.73 and 1.20 to 14.30 mg P kg^{-1} soil as the inorganic and organic form, respectively.

All amendments increased organic P in the less available P fraction in both soils, whereas residual P was increased with regards to the control only by PM amendments.

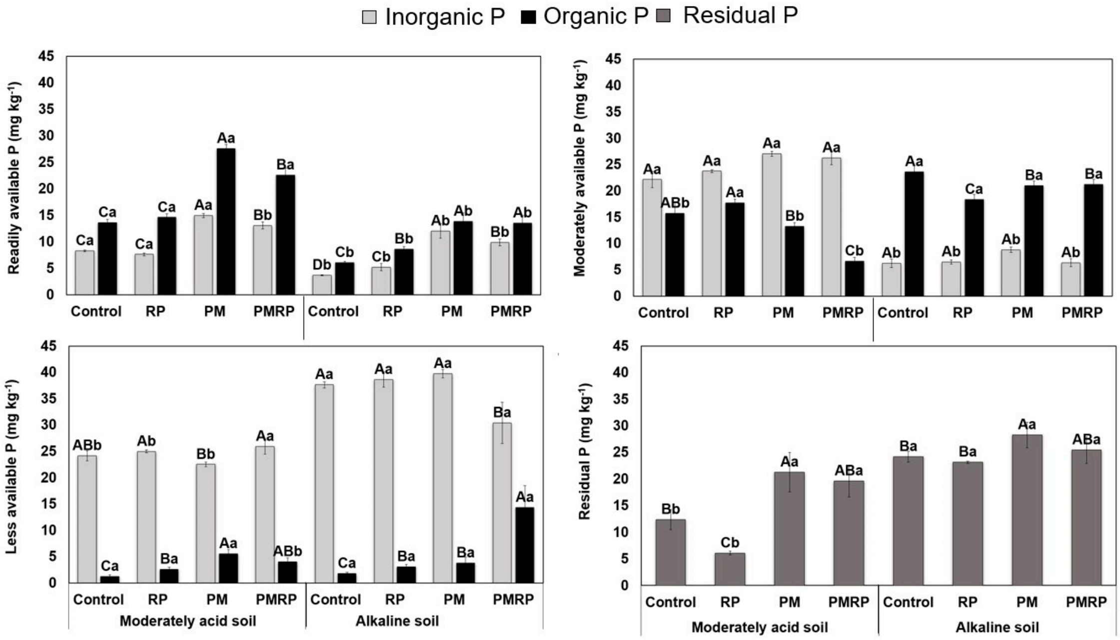

Figure 1. Inorganic and organic P concentration of fractions extracted from control and soil amended with poultry manure compost (PM), phosphate rock (RP), and their combination (PMRP) after 7 weeks of ryegrass growth. Upper case letters denote significant differences ($p \leq 0.05$) between treatments for one soil. Lower case letters denote significant differences ($p \leq 0.05$) between soils for one treatment.

3.3. Microbial Biomass P

Microbial biomass P ranged between 7.55 to 35.10 mg P kg^{-1} soil, and it was significantly increased with all P amendments as compared to the control (Figure 2). With PM amendment, microbial biomass P concentrations increased greatly as compared to RP amendment by 81% to 93% in moderately acid and alkaline soil, respectively (Figure 2). In the moderately acid soil, PM and its combination with RP induced the greatest increases of microbial biomass P (81–100%) as compared with RP while for the alkaline soil, only PM as a single amendment increased MBP with regards to RP.

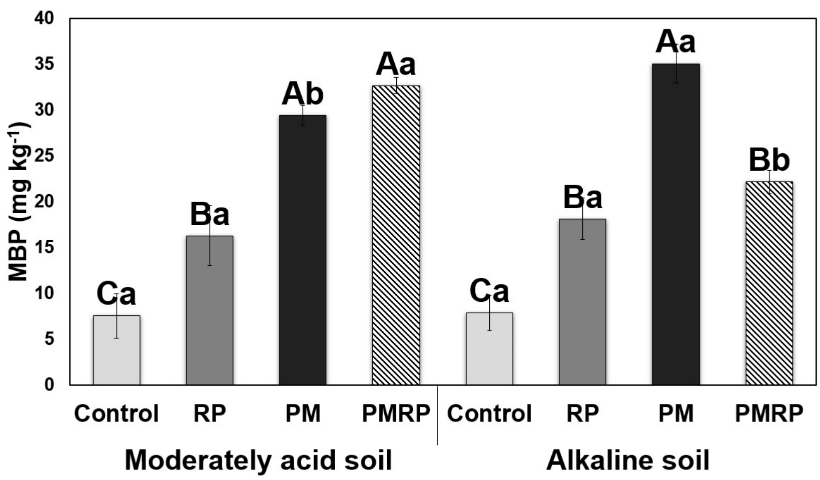

Figure 2. Microbial biomass phosphorus (MBP) in in control and soil amended with poultry manure compost (PM), phosphate rock (RP), and their combination (PMRP) after 7 weeks of ryegrass growth. Upper case letters denote significant differences ($p \leq 0.05$) between treatments for one soil. Lower case letters denote significant differences ($p \leq 0.05$) between soils for one treatment.

3.4. Shoot and Root Biomass Production

Plants grown in the moderately acid soil showed greater root biomass in all treatments compared to those grown in the alkaline soil, whereas shoot biomass was similar for both soils, except for the RP treatment.

The dry weight of plants cultivated in moderately acid soil treated with different P amendments and their combination was significantly higher ($p \leq 0.05$) compared to the control (Figure 3). The PM and RP increased shoot biomass similarly (~2.5 fold) as compared with the control. Root biomass increased by 1.2 to 3.3-fold in both soils treated with either PM alone or combined with RP.

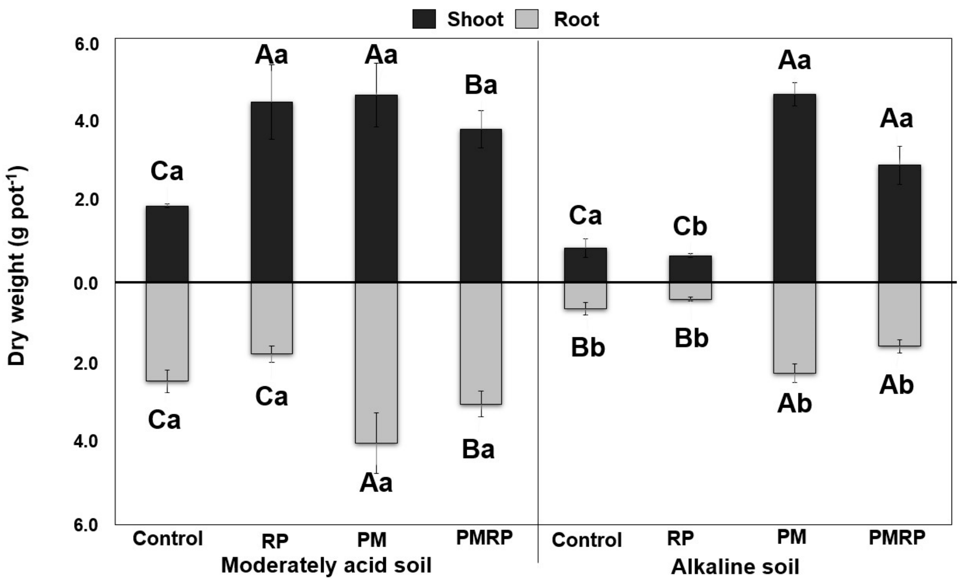

Figure 3. Dry weight of shoots and roots in control and soil amended with poultry manure compost (PM), phosphate rock (RP), and their combination (PMRP) after 7 weeks of ryegrass growth. Upper case letters denote significant differences ($p \leq 0.05$) between treatments for one soil. Lower case letters denote significant differences ($p \leq 0.05$) between soils for one treatment.

In the alkaline soil, no difference in dry matter production was observed for the RP treatment with respect to the control. In contrast, PM alone and its combination with RP significantly increased shoot biomass (5.6 and 3.5-fold) and root biomass (3.3 and 2.3-fold) as compared to the control (Figure 3).

3.5. Shoot and Root P Concentrations and Uptake

Shoot and root P concentrations and root P uptake increased significantly with all amendments as compared to the control (Table 4). Shoot P uptake showed no change compared to the control for both soils. The highest increase was noted for PM and PMRP treatments, which showed on average 37% to 48% higher root and shoot P concentrations than plants of the control treatment (Table 4). This is in line with a higher P uptake (Table 4). Shoot P concentration and uptake was higher in all treatments except RP in the moderately acid soil as compared to the alkaline one. Despite differences in uptake, root P concentrations were similar for both soils.

Table 4. P concentration and uptake of roots and shoots and P use efficiency after 7 weeks of ryegrass growth in control soil and soil amended with poultry manure compost (PM), phosphate rock (RP), and their combination (PMRP).

Soil Type	Treatment	P conc		P uptake		P Use Efficiency
		Shoot	Root	Shoot	Root	
		g kg^{-1}		mg		% of input
Moderate acid	Control	7.2 ^{Da}	1.4 ^{Da}	3.6 ^{Ba}	3.6 ^{Ca}	-
	RP	12.5 ^{Ca}	2.2 ^{Ca}	3.5 ^{Ba}	4.6 ^{Ca}	6 ^{Ca}
	PM	24.2 ^{Aa}	3.8 ^{Aa}	4.9 ^{Aa}	14.8 ^{Aa}	28 ^{Aa}
	PMRP	16.6 ^{Ba}	2.8 ^{Ba}	5.0 ^{Aa}	8.7 ^{Ba}	14 ^{Ba}
Alkaline	Control	1.4 ^{Db}	1.7 ^{Ba}	2.6 ^{Bb}	1.1 ^{Cb}	-
	RP	4.9 ^{Cb}	2.1 ^{Ba}	3.0 ^{Ba}	2.8 ^{Ba}	5 ^{Ca}
	PM	17.7 ^{Ab}	3.3 ^{Aa}	3.8 ^{Ab}	9.7 ^{Ab}	25 ^{Aa}
	PMRP	11.3 ^{Bb}	3.0 ^{Aa}	3.8 ^{Ab}	6.9 ^{Aa}	16 ^{Ba}

Upper case letters denote significant differences ($p \leq 0.05$) between treatments for one soil. Lower case letters denote significant differences ($p \leq 0.05$) between soils for one treatment.

Plant P use efficiency of the added P sources and their combinations ranged from 5% to 6 % for RP to a maximum of ~28% for PM alone (Table 4). Plant use efficiency of the mixture, PMRP, was in between these values. Few differences were noted between soils.

3.6. Relationship between Soil and Plant Parameters

In the alkaline soil, the readily available inorganic and organic P fractions, moderately available Po, residual P, and microbial biomass P were strongly correlated with shoot and root biomass and nutrient uptake (Table 5).

Table 5. Relationship between soil and plant parameters after 7 weeks of ryegrass growth in control and soil amended with poultry manure compost (PM), phosphate rock (RP), and their combination (PMRP). *denotes significant correlation coefficients ($p \leq 0.05$), $n = 16$.

Soil	Soil Parameters	Shoot Biomass	Root Biomass	P Uptake	
				Shoot	Root
Moderately acid soil	readily-Pi	0.41	0.80 *	0.87 *	0.92 *
	readily-Po	0.51 *	0.78 *	0.95 *	0.96 *
	moderately-Pi	0.61 *	0.59 *	0.83 *	0.83 *
	moderately-Po	−0.07	−0.43	−0.37	−0.39
	Less avail.-Pi	−0.10	−0.44	−0.40	−0.45
	less-avail. Po	0.71 *	0.61 *	0.93 *	0.89 *
	Residual-P	0.19	0.70 *	0.63 *	0.74 *
	Microbial P	0.62 *	0.61 *	0.83 *	0.78 *
	Total soil N	0.65 *	0.69 *	0.94 *	0.91 *
	Total soil C	0.52 *	0.71 *	0.89 *	0.88 *
Alkaline soil	readily-Pi	0.94 *	0.93 *	0.96 *	0.91 *
	readily-Po	0.84 *	0.82 *	0.89 *	0.84 *
	moderately-Pi	0.75 *	0.67 *	0.75 *	0.67 *
	moderately-Po	0.09	0.16	−0.18	−0.13
	Less avail.-Pi	−0.03	−0.14	−0.03	−0.09
	less-avail. Po	0.30	0.38	0.33	0.39
	Residual-P	0.81 *	0.80 *	0.70 *	0.66 *
	Microbial P	0.88 *	0.84 *	0.95 *	0.88 *
	Total soil N	0.45	0.31	0.60 *	0.51
	Total soil C	0.81 *	0.80 *	0.82 *	0.77 *

In the acid soil, inorganic and organic P in the readily available fraction and moderately available-Pi showed a positive correlation with root biomass and root P uptake (Table 5). Residual P was correlated with root biomass and less available organic P was somewhat correlated with shoot biomass and root P uptake. In this soil, the strongest correlations were noted for P uptake in the root and shoot with readily available P, moderately available Pi, less available Po, microbial P, total C, and total N.

To investigate the effect of the different types of amendments on soil and plant parameters, we performed PCA analyses. The first two PCA components explained 82% of the total variance of soil P fractions (Figure 4A). The individual representation of treatments on the factor map showed spatial separation of treatments in both soils, with a tendency to the formation of two groups, one for the PM and PMRP treatments and a second one for the control and RP (Figure 4A). Both groups were separated along the 2nd axis related to the contribution of less available organic P and residual P. Additionally, acid and alkaline soils could be separated according to soil P forms along the first axis. Alkaline soils were thus associated with less and moderately available Po and less available Pi, while acid soils were associated with readily available Pi and Po and moderately available Pi (Figure 4A).

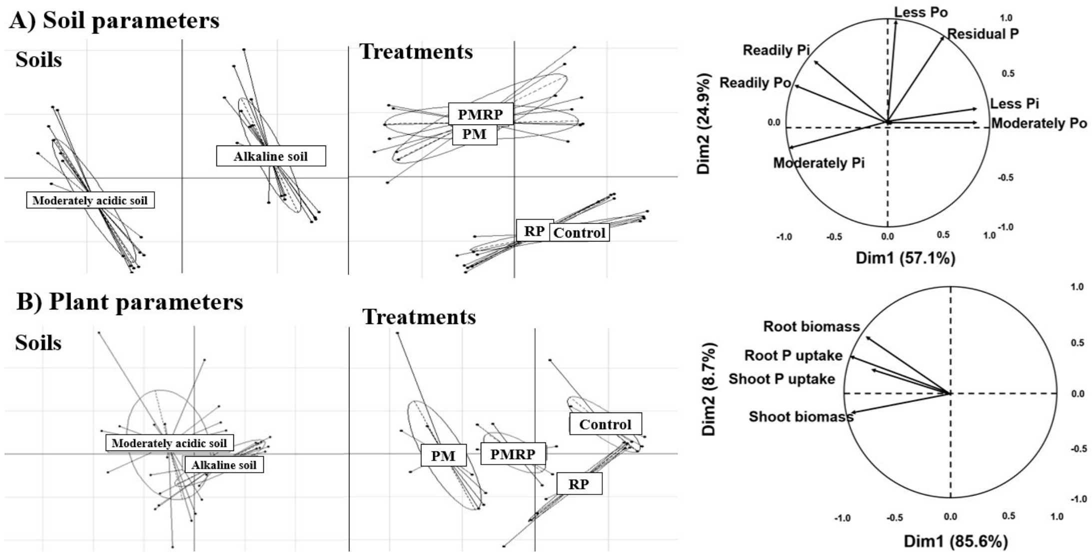

Figure 4. Principal component analysis of soil P forms (**A**) and plant (**B**) variables studied as the response to different soil types and treatments.

For the PCA performed with plant parameters, the first two components explained 94% of the total variability. The individual representation of treatments on the factor map (Figure 4B) showed spatial separation of all the treatments of both soils. Treatments with poultry manure were associated with P uptake and aboveground and belowground biomass in the positive direction. Plant parameters did not differentiate acid and alkaline soils.

3.7. Synergistic and Antagonistic Effects between PM and RP on Soil and Plant Parameters

To elucidate the effects of the amendment mixture, PMRP, on plant and soil parameters, we compared the expected values as the sum of the single use of PM and RP to the observed value for the mixture. Differences were interpreted in terms of synergistic and antagonistic effects. While the mixture had a similar synergistic effect on P availability in both soils, their effect on plant parameters was strongly dependent on the soil type (Table 6). For the alkaline soil, the mixture had no or a slightly antagonistic effect on plant biomass and P uptake, while in the moderately acid soil, the mixture had an antagonistic effect on P uptake, and a synergistic effect on root and shoot biomass.

Table 6. Synergistic and antagonistic effect on soil and plant parameters as the response of the combined application of poultry manure compost and phosphate rock applied at a P rate of 100 mg kg^{-1} soil after 7 weeks of ryegrass growth. +SD.

			Observed	Expected	Mixture Effect (%)
Moderately acid soil	Soil	P availability (mg kg^{-1})	137.1 ± 21.1	132.5 ± 4.9	9.9 ± 1.8
	Shoot	Biomass (g)	1.9 ± 0.5	1.6 ± 0.4	25.5 ± 3.9
		P uptake (mg)	9.4 ± 1.3	12.3 ± 1.2	−23.6 ± 1.8
	Root	Biomass (g)	1.16 ± 0.3	0.65 ± 0.2	91.3 ± 4.9
		P uptake (mg)	1.5 ± 1.1	3.97 ± 0.9	−59.6 ± 3.7
Alkaline soil	Soil	P availability (mg kg^{-1})	133.5 ± 15.0	114.4 ± 9.5	17.5 ± 3.0
	Shoot	Biomass (g)	2.1 ± 0.5	2.38 ± 0.2	−13.1 ± 1.9
		P uptake (mg)	9.97 ± 0.3	11.6 ± 0.5	−13.6 ± 2.6
	Root	Biomass (g)	0.78 ± 0.1	0.81 ± 0.1	−3.3 ± 1.2
		P uptake (mg)	5.5 ± 2.1	5.82 ± 1.2	1.8 ± 0.2

4. Discussion

4.1. Impact of Organic and Inorganic P Amendments on C, N, and P Stoichiometry and Microbial Biomass P

Treatments with poultry manure compost (PM and PMRP) showed increased soil C, N, and P concentrations, most probably related to organic matter input through the amendment as well as higher biomass production (Figure 3). All amendments changed to some extent soil organic matter stoichiometric ratios. These ratios determine the interlinkage between biochemical cycles of C, N, and P, providing information about nutrient availability following SOM decomposition and stabilization processes. The effect of the amendments on stoichiometric ratios was dependent on the type of amendment and also the soil type. Differences in stoichiometric ratios in the two soil types suggest that the amendment effect could be soil pH dependent.

Amendments increased P incorporation into the microbial biomass. Soil type had an effect on the microbial response to the application of the mixture (PMRP). It was interesting to note that while in the moderately acid soil, microbial biomass P was similar in PM and PMRP, in the alkaline soil, microbial biomass P was lower in PMRP compared to PM (Figure 2). Similar negative effects of inorganic fertilizers on soil microorganisms have been reported before [25]. The results of this study show an antagonistic effect of both materials when applied in combination to alkaline soil. In view of the importance of soil microorganisms for the maintenance of soil fertility and the sustainability of grassland ecosystems and their role for P immobilization, especially in soils with low C:P ratios, such as the ones of the present study [26], we suggest that the soil reaction may be an important criterion to consider when organic P fertilizers are applied in combination with inorganic ones.

4.2. Impact of Organic and Inorganic P Amendments on Nutrient Uptake and Biomass Production and Soil P Forms

Our results, indicated that shoot biomass increased with all P sources, except for RP in alkaline soil (Figure 3). A contrasting effect of RP on plant growth depending on the soil reaction has been observed before and the higher efficiency of RP under acid soil conditions is well documented (e.g., [18]). As many studies have shown [12], biomass production was strongly correlated to readily available inorganic P (r = 0.94 for shoot biomass in the alkaline soil and for root biomass in both soils) (Table 6). Readily available soil P may be already present in amendments [27] or may have been mineralized from organic forms after amendment addition [28]. Our data indicate that while large amounts of readily available P were added with PM (Table 2), in both soils, P uptake was correlated to microbial biomass P, less available organic P, and/or residual P, C, and N (Table 5). This suggests that the mineralization of organic matter is an important process for biomass production after amendment addition. Soil reaction may influence the importance of the latter process, as much stronger correlations between those parameters were noted in the acid soil as compared to the alkaline one. The importance of the soil reaction for soil P forms is further illustrated by the PCA analyses of soil parameters (Figure 4A).

However, this is different for plant parameters, which were differentiated by treatments, but not soil types (Figure 4B).

Our data show that using 14 Mg ha^{-1} PM as a single amendment supplied sufficient nutrients to meet plant requirements well above the critical N, P, and K concentration in shoots for producing 90% of the maximum ryegrass yield, which are 18, 3.4, and 28 mg g^{-1} [10]. Since PM is a very rich animal manure compost, it may help to build up soil productivity better than other amendments due to additional effects [29]. This is especially true for the moderately acidic soil, which was not deficient in plant available P as indicated by the plant P concentrations of the control treatment. However, we found that in both soils, PM addition increased the shoot and root P uptake as compared to the RP when used as single amendments (Table 4). Moreover, PM led to higher plant P use efficiency than RP (Table 4). This could be an indication that there are nutrient limitations other than N, P, and K occurring, which were counterbalanced with PM. Moreover, it is also possible that PM led to the stimulation of microbial activity by promoting P uptake [21].

4.3. Synergistic and Antagonistic Effects of the Combined Application of PM and RP

Our results showed that combining RP with PM may be highly efficient in increasing the soil available P concentrations, thereby enhancing biomass production. While, in general, RP has low concentrations of available P [30], various studies [31–34] have demonstrated that P availability from RP may be increased by co-application of organic amendments. For example, Sohail et al. [35] showed that combining RP with compost increases soil P availability, and Abbasi and co-workers [18] demonstrated that the release of P to the soil increased by 80% compared with when RP was combined with PM.

In this study, our data revealed strong synergistic effects in terms of P availability independent of the soils' pH (Table 6). In contrast, biomass production showed a strong synergistic response in the moderately acid soil and an antagonistic response in the alkaline soil. Soil type dependent antagonistic effects were also observed for P uptake. Root P uptake was lower than expected in the moderately acid soil, while it was similar to the expected value in the alkaline soil (Table 6). This might be explained by a higher immobilization of P in the microbial biomass in moderately acid soil as compared to the alkaline one (Figure 3). Microbial P may become available and thus microbial immobilization as well as the antagonistic effect might be transitory. Another explanation of these contrasting effects may be related to the initial P status of the soil. As the moderately acid soil was not P deficient, the increased availability of P following PM and RP did not foster uptake. We suggest that synergistic and antagonistic effects in different soil types should be evaluated and taken into consideration, when elaborating new fertilizer strategies through the combination of organic and inorganic fertilizers.

5. Conclusions

Our study showed that the P distributed between inorganic and organic P fractions was greatly affected by the type of amendment and soil type. Poultry manure compost increased highly soil P availability, consequently improving above and belowground plant biomass production on both soil types, while phosphate rock amendment had limited effects on soil P fractions and positive effects on aboveground plant biomass production only in moderately acidic soil. In general, the influence of amendment type on soil parameters was limited and mainly related to the organic matter input. In contrast, the soil amendment type had a strong effect on plant parameters.

We found synergistic effects of the combined use of PM and RP for soil available P in both soils. For plant parameters, synergistic and antagonistic effects were soil type dependent. We therefore suggest that fertilizer strategies through the combination of organic and inorganic fertilizers must be tested in different soil types by quantifying their synergistic and antagonistic effects. Moreover, the use of poultry manure compost, alone or combined with phosphate rock, could be a strategy to replace inorganic fertilizers and should be tested in long-term field experiments.

Author Contributions: Data curation, P.P.-G.; Formal analysis, P.P.-G.; Funding acquisition, C.R., P.C. and M.d.L.L.M.; Investigation, C.R.; Methodology, P.B. and T.B.; Resources, T.B. and C.R.; Software, P.B.; Supervision, M.d.L.L.M. and C.R.; Visualization, P.P.-G.; Writing—original draft, P.P.-G.; Writing, review, and editing, P.P.-G., P.B., P.C., M.d.L.L.M. and C.R.

Funding: This research was funded by CNRS under the framework of the EC2CO project LOMBRICOM, FONDECYT projects 1181050 and 1161326, and Conicyt scholarship n° 21150715.

Acknowledgments: The authors gratefully acknowledge the IEES laboratory for technical support. We also thank the SOERE-PRO for providing the soils used in this study and the KOMECO company for providing the poultry manure compost.

Conflicts of Interest: The authors declare no conflict of interest.

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