

Effect of Millet Growth, N Sources and Previous Phosphorus Availability on the Efficiency of P Sources

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Abstract

The efficiency of phosphate fertilization has been a major challenge for tropical agriculture. Lately, cover plants better adapted to tropical conditions have allowed non-tillage in these areas. Our aim was to investigate the effects of soil and plant (millet) on the efficiency of different P sources combined with N sources in an oxisol with medium and low initial P availability. For this, rhizotubes filled with oxisol under different combinations of P and N source were exposed to the absence and presence of millet plant (*Pennisetum glaucum* L., Leke). We characterized the soil before and after, as well the fertilizer sources and plants. There was no effect of medium or low initial P availability in the soil on the P recovery rate (PRR). NH_4^+ was advantageous mainly combined with a soluble P source, increasing up to 40% the P recovery rate in relation to NO_3^- . Bayóvar phosphate rock (PR) had higher solubilization than Araxá PR, as well as a significant soil pH increase. Without plants, there was no significant correlation among the variables evaluated, evidencing the plant's role in dissolving less soluble P sources in an oxisol. Our findings suggest no effect of previous medium P availability on the P recovery rate regardless of phosphorus sources. In addition, an improvement in the P recovery rate when an ammoniacal source is used is observed when soluble phosphate fertilizers are used. Cover plants on oxisols are crucial for increasing the efficiency of phosphorus fertilizers and their plant availability over time.

Keywords: oxisol, millet, rhizosphere, phosphate rocks, nitrogen

1. Introduction

The consumption of P fertilizers has increased during recent decades in Brazil (Withers et al., 2018). About 5 million metric tons of phosphate (P_2O_5) fertilizers are used each year in Brazilian agriculture (IPNI, 2016), making the country the fourth highest consumer of P fertilizer worldwide (Liebert, Griswold, & Wilson III, 2015). In addition, the country is one of the largest consumers of natural phosphates *in natura* (IFA, 2013). However, P fertilization in tropical soils is known to be very inefficient (Roy et al., 2016), due to strong retention of phosphates by chemisorption on Fe- and Al-oxyhydroxides or precipitation into P-insoluble minerals (Novais & Smyth, 1999; Parfitt, 1979).

The fates of the P added as fertilizer to crops are affected by several chemical properties of soils. In highly weathered soils, even large additions of P as fertilizer can be retained by soil particles, it being unavailable for plant uptake. In these conditions, recovery by crops of the P added can be less than 10% (Baligar & Bennett, 1986; Santos et al., 2017). Soil management which keeps cover plants over time is capable of increasing P availability. In fact, covered soil has greater potential to improve the cycling of P in soil/plant systems (Calonego & Rosolem, 2013), and also to prevent its fixation because of the organic matter dynamic of the soil (Guppy et al., 2005; Hunt et al., 2007). Brazil has extensive no-tillage areas, occupying about 32 million ha (FEBRAPDP/CONAB, 2012), in which the cultivation of millet (*Pennisetum glaucum* L.) has increased as a crop rotation due to its high biomass yield and robust root system, even under higher temperatures.

The continued application of phosphate sources over time, and the use of cover plants better adapted to no-till farming can alter the dynamics of P in soil to increase its plant availability (Teles et al., 2017). In fact, the availability of phosphate is affected by the root system architecture, and modifications to the rhizosphere chemical environment are of crucial importance for efficient management of phosphate fertilization (Dakora & Phillips, 2002; Hinsinger, 2001; Hinsinger & Gilkes, 1996). On the other hand, combinations of phosphate sources with ammoniacal sources have shown promise in calcareous soils (Hinsinger & Gilkes, 1996; Jing, Rui, Zhang, Rengel, & Shen, 2010) but with little information for tropical soils.

Despite the toxicity caused by a high concentration of N-NH_4^+ in most plant species (Esteban, Ariz, Cruz, & Moran, 2016; Kronzucker, Siddiqi, & Glass, 1997), the majority of studies predict an increase in rhizospheric acidity and greater P uptake with N-NH_4^+ application, while results with N-NO_3^- application have shown the opposite trend (Hoffmann, Ladewig, Claassen, & Jungk, 1994; Kant, Peng, & Rothstein, 2011; Sarkar & Wyn Jones, 1982). The supply of N-NO_3^- to ryegrass resulted in little acidification of the rhizosphere and dissolution of about 20% of the PR added; however, when N-NH_4^+ was supplied, there was a marked increase in rhizosphere acidity followed by an increase in rock dissolution (38% of total P) (Hinsinger & Gilkes, 1996). In maize cultivation in limestone soils, there was a significant improvement with the localized application of P combined with ammonium, favoring the growth and utilization of nutrients in the early stages, as well as root proliferation and acidification of the rhizosphere (Jing et al., 2010). Also in maize crop, N-NH_4^+ induced acidification and optimized acid phosphatase activity excreted by roots, which improved root growth and P absorption of phytin and NH_4^+ (Ding et al., 2011).

Thus, the objective of this study was to investigate the effects on soil and plants of different P sources (with different reactivity) combined with N sources (N-NH_4^+ or N-NO_3^-) on an oxisol with different initial P availability (low and medium).

2. Material and Methods

2.1 Soil Sampling

Soil samples were collected from a top layer (5-20 cm) of a Red-Yellow Oxisol in the municipality of Viçosa, Minas Gerais, Brazil. The soil was air-dried and passed through a sieve (2 mm) screen. Briefly, the soil had 63% clay, pH_{water} 4.5, CEC 5.9 $\text{cmol}_c \text{ dm}^{-3}$, 5% base saturation, OM 17 g kg^{-1} , P Mehlich⁻¹ 1.2 mg dm^{-3} , and P-remaining of 8 mg dm^{-3} .

Aiming to increase the base saturation of the soil to 50%, the soil samples were put into plastic bags, mixed with lime ($\text{CaCO}_3:\text{MgCO}_3$ at a Ca/Mg ratio of 3.5:1), wetted to 80% of the field capacity and maintained at that level for 30 days. After this time, part of the soil was mixed with triple superphosphate fertilizer (TSP, 150 mg dm^{-3} P) and incubated for 30 more days, in order to increase its P availability. After incubation with TSP, soil samples were air-dried and passed through a sieve (2 mm) screen for chemical characterization. The results showed small differences in pH (5.6 and 5.4), Ca (2.45 and 2.35 $\text{cmol}_c \text{ dm}^{-3}$) and Mg (0.43 and 0.6 $\text{cmol}_c \text{ dm}^{-3}$), but not P-resin (0.07 and 30 mg dm^{-3}), before and after their incubation with TSP, respectively.

2.2 Characterization of P and N Sources

We used as P sources TSP fertilizer (44.0% P_2O_5), Bayóvar PR (29.8% of total P_2O_5 and 15.6% soluble P_2O_5 in 2% citric acid) and Araxá PR (30.7% of total P_2O_5 and 4.6% soluble P_2O_5 in 2% citric acid). As N-NH_4^+ source, we used $(\text{NH}_4)_2\text{SO}_4$, and for N-NO_3^- , $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ was used. For additional treatments, 9 mg dm^{-3} of dicyandiamide (DCD) was used as a nitrification inhibitor agent (Di & Cameron, 2004).

2.3 Trial Design and Conduct

For confined root growth and greater soil exploration, cylindrical rhizotubes were made of chlorinated polyvinyl chloride (CPVC), 1.16 cm in diameter and 15.12 cm in length. The tubes were fixed onto a polyvinyl chloride (PVC) plate to be suspended. Each rhizotube was filled with 10 cm^3 of soil sample. At the lower end of the rhizotubes, a 0.20-mm nylon screen was glued, to contain the soil. Plant-free rhizotubes were used as a control treatment for comparison and better understand the plant's role.

The P and N fertilizers were previously mixed with each other and applied to the soil volume. A pre-germinated plant of millet (*Pennisetum glaucum* L., Leeke) was cultivated in each rhizotube for 46 days in a growth chamber, with a photoperiod of 12 h, at 26 ± 2 °C and radiance of $130 \pm 5 \mu\text{mol m}^{-2} \text{ s}^{-1}$.

The treatments were arranged in a $2 \times 3 \times 4$ factorial scheme, with two initial P levels in the soil (low and medium); three P sources (Bayóvar PR, Araxá PR and TSP) at a dose of 300 mg dm^{-3} of P; four N sources: a control treatment (0 mg dm^{-3} N), 70 mg dm^{-3} of N-NH_4^+ [$(\text{NH}_4)_2\text{SO}_4$ without and with DCD], and N-NO_3^- [$\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$].

Other nutrients were supplied daily through a solution, totaling 160 mg dm⁻³ K (KCl), 66 mg dm⁻³ S (K₂SO₄), 2.00 mg dm⁻³ Zn (ZnCl₂), 1.83 mg dm⁻³ Mn (MnCl₂·4H₂O), 0.78 mg dm⁻³ Fe (FeCl₃·6H₂O), 0.67 mg dm⁻³ Cu (CuCl₂·2H₂O), 0.40 mg dm⁻³ B (H₃BO₃) and 0.08 mg dm⁻³ Mo ((NH₄)₆Mo₇O₂₄·4H₂O).

2.4 Sampling and Chemical Analysis

The plants and soil samples were collected 46 days after transplanting. The soil was separated from the roots, homogenized, air-dried and sieved (2 mm sieve). Samples of shoots and roots were oven-dried at 65±2 °C for 72 h until constant mass, weighed and milled for chemical analysis. We kept the original soil samples, which were considered as references (T₀).

The plant samples were mineralized in an open-vessel digestion system using a nitric-perchloric solution (4:1 v v⁻¹) (Miller, 1998). The N content was measured by the Kjeldahl method, P by molecular absorption spectroscopy, and Ca and Mg by atomic absorption spectroscopy.

The soil samples were analyzed for pH in water, and available content of Ca²⁺, Mg²⁺ (EMBRAPA, 1997), NH₄⁺ (Kempers & Zweers, 1986), NO₃⁻ (Yang, Kim, Skogley, & Schaff, 1998) and P (Raij, Quaggio, & Silva, 1986).

2.5 Data and Statistical Analysis

The total P content was obtained from the product of dry matter mass and its concentration in the tissues (shoot or root) of the millet. The P recovery rate (PRR) by plants was calculated by Equation 1:

$$PRR = [(AM - AMC)/(AAP)] \times 100 \quad (1)$$

where,

AM: amount of P accumulated in the plant tissue when P fertilizers were used; AMC: amount of P accumulated in the plant tissue for the control treatment; AAP: amount of added P as fertilizer, corresponding to 300 mg dm⁻³ P from the different sources.

Solubilization of the PR was evaluated by the exchangeable Ca content in the soil and Ca concentration in the plant tissues (adapted from Bolan & Hedley, 1990) by Equation 2:

$$PR \text{ solubilization} = \Delta Ca = [(Ca_{T46} + Ca_{plant}) - Ca_{T0}] \quad (2)$$

where,

Ca_{T46}: quantity of Ca in the volume of soil used at the end of 46 days of millet cultivation (mg rhizotube⁻¹); Ca_{plant}: Ca content in the plant at the end of 46 days of millet cultivation (mg plant⁻¹); Ca_{T0}: quantity of Ca in the volume of soil used in T₀ (mg rhizotube⁻¹).

Initially, the normality of the errors and the homogeneity of variance were verified. Nonconforming data were transformed according to a Box and Cox approach (Box & Cox, 1964). Statistical analysis was carried out by three-way analysis of variance (ANOVA) using a general linear model through GENES and R software (Cruz, 2013; R Development Core Team, 2016). The effects of the P sources with each combination of N and within each P level were evaluated by the Duncan test at 5% significance. Pearson's correlations were also calculated. Analyses were performed using Sigma Stat software v.2.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 7 (GraphPad Prism version 7 for Windows, GraphPad Software, La Jolla, California, USA). In addition, we applied the false discovery rate (FDR)-controlling method for each correlation p-value (Benjamini & Hochberg, 1995), using an R script in Rbio software (www.biometria.ufv.br) (Bhering, 2017; R Development Core Team, 2016).

3. Results

3.1 P Recovery Rate and Biomass

By decomposing the factorial, we can observe in detail the effect of the combinations of P and N sources on the P recovery rate, PRR (Figure 1). There were no significant differences in PRR by millet contrasting the initial P availability; nevertheless, the performance of the P or N sources was affected by it. PRR followed the solubility of the P sources. TSP provided higher values, followed by Bayóvar PR and Araxá PR. Interactions between TSP and ammonium sulfate promoted a higher PRR than other treatment combinations. The combination of TSP with the nitric source (TSP+N) provided a PRR similar to that for no N supply. In addition, when TSP was combined with N-NO₃⁻, the PRR was 40% lower than when it was associated with the ammoniacal nitrogen source.

For the PRs (Bayóvar PR and Araxá PR), there were no differences in terms of PRR for their combinations with the nitrogen sources. However, one treatment that drew attention was Bayóvar PR combined with ammonium

sulfate plus DCD in soil with medium initial P availability. This treatment was the only one that differed from the control.

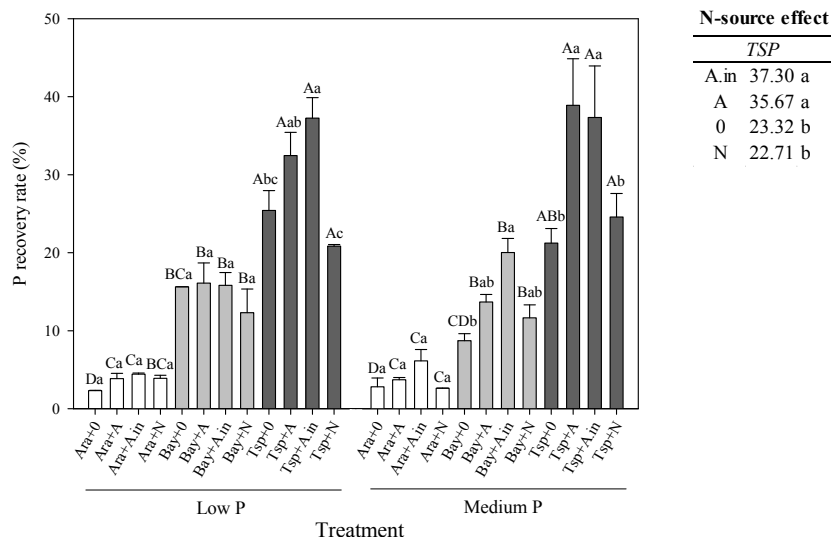


Figure 1. P recovery rate (PRR) by millet grown under 24 different combinations of P and N sources. The factorial “P source × N source” was splitted and shown in the figure. P sources: Ara = Araxá PR, Bay = Bayóvar PR, TSP = triple superphosphate. Nitrogen sources: 0 = non-application of N, A = ammonium sulfate, A.in = ammonium sulfate with dicyandiamide (DCD) – a nitrification inhibitor agent, N = magnesium nitrate. Uppercase letters compare the means between the P sources within each N source. Lowercase letters compare the N sources within each P source. Means were compared by the Duncan test at 5% significance

There were no differences in shoot or root production by millet when the initial P availability was low or medium (Figure 2). However, in soil with low initial P availability, the ammoniacal source plus DCD provided greater shoot dry weight production compared to that with the nitric source, but equivalent to that with ammonium without DCD. In general, the similar values of shoot and root dry weight suggest that the greatest PRR was mainly due to an increase in the P content in plant tissues, and not to the increase in biomass.

The combination of Araxá PR with the ammoniacal source plus DCD, when the initial P availability was low, significantly increased its performance as fertilizer. This combination was as efficient as other combinations in terms of shoot dry weight production by millet (Figure 2).

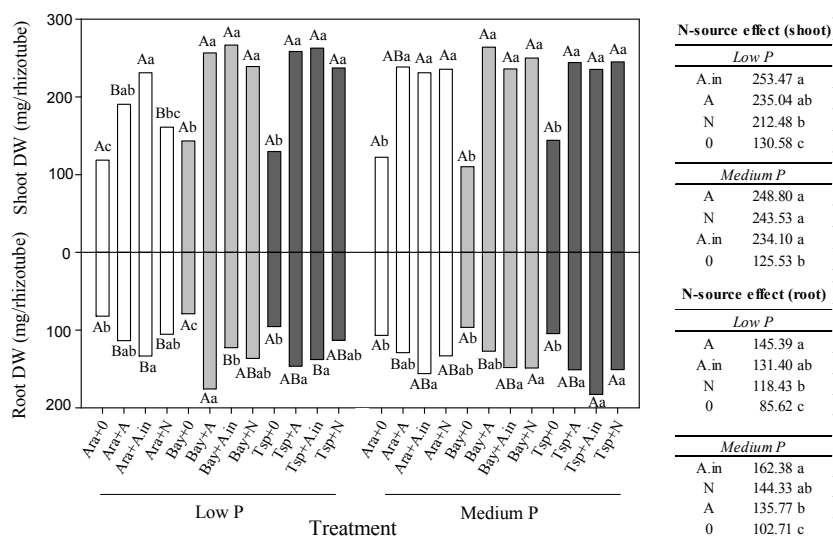


Figure 2. Shoot and root dry weight of millet grown under 24 different combinations of P and N sources, at low and medium initial P level. The factorial “P source \times N source” was splitted and shown in the figure. P sources: Ara = Araxá PR, Bay = Bayóvar PR, TSP = triple superphosphate. N sources: 0 = non-application of N, A = ammonium sulfate, A.in = ammonium sulfate with dicyandiamide (DCD) – a nitrification inhibitor agent, N = magnesium nitrate. Uppercase letters compare the mean between the P sources within each N source. Lowercase letters compare the N sources within each P source. Means were compared by the Duncan test at 5% significance. The table on the right shows the interaction of “N source \times initial P level” in the shoot and root dry weight

3.2 Solubilization of Phosphate Rocks (ΔCa) and P Variation in the Rhizospheres (ΔP)

Solubilization of the PR was not affected by the initial P availability in soil (Figure 3). For both P availabilities, Bayóvar PR was more soluble than Araxá PR. In general, when N was not supplied, we observed smaller ΔCa than in its presence, demonstrating greater solubilization of PRs when N was used. In soil with low initial P availability, the combination of Araxá PR with the ammoniacal source and DCD provided a greater ΔCa in relation to the combination with the nitric source. Interestingly, in soil with medium initial P availability, there were higher ΔCa when Araxá PR was combined with the nitrate source. Unlike that of Araxá PR, the solubilization of Bayóvar PR was affected by the N supply only in soil with low initial P availability, without differences between the N sources.

ΔP is a variable complementary to ΔCa to predict the solubilization of PRs in soil, but it is less predictive (Figure 4). Indeed, in oxisols, the solubilization of PRs does not always correlate with the plant P availability because of the fast kinetics of P chemisorption on Fe- and Al-oxyhydroxides compared to its release rate from PRs. The ΔP value was higher in soils with medium P availability. Bayóvar PR provided a greater amount of P in the soil in relation to Araxá PR, about 2.5-fold more. There was a significant effect of the combination of Bayóvar PR and the ammoniacal source. By decomposing the factorial, we observed that this effect was most pronounced in soil with medium initial P availability.

3.3 Multivariate Analysis

Principal component analysis (PCA) indicated a separation of the combinations of the P and N sources (Figure 5). The first major component (PC1) explained 52.1% of the variation. The main loading factors that contributed to the separation in PC1 were P content in roots, PRR, and P and Ca content in shoots.

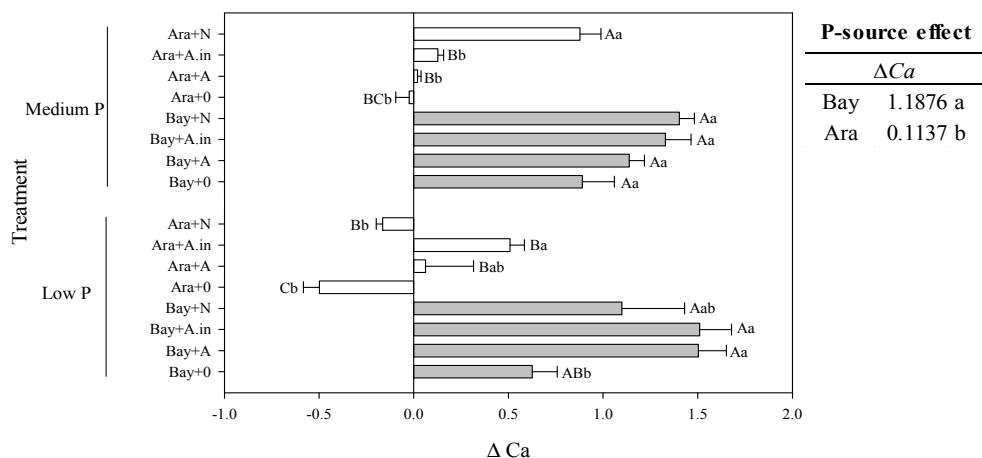


Figure 3. ΔCa of millet rhizosphere soil cultivated under 16 different combinations of PR and N sources, at low and medium P level. The factorial “P source \times N source” was splitted and shown in the figure. P sources: Ara = Araxá PR, Bay = Bayóvar PR, TSP = triple superphosphate. N sources: 0 = non-application of N, A = ammonium sulfate, A.in = ammonium sulfate with dicyandiamide (DCD) – a nitrification inhibitor agent, N = magnesium nitrate. Uppercase letters compare the mean between the P sources within each N source. Lowercase letters compare the N sources within each P source. Means were compared by the Duncan test at 5% significance. The table on the right shows the interaction of P-source effect in ΔCa

(Figure 5B, Table A1). The best treatments in relation to the variables evaluated are to the right of the y-axis in Figure 5A. The most promising combinations included TSP fertilizer as the P source, which is highly water-soluble. In soil with low initial P availability, the effectiveness of TSP when it was associated with ammonium sulfate was highlighted. Overall, Bayóvar PR and Araxá PR were less effective than TSP, respectively. Apparently, when considering all variables analyzed, for TSP and Bayóvar PR, combination with the ammoniacal source was more effective when the initial P availability was low. Under medium initial P availability, the N supply was important but there were few differences between the N sources. This same behavior was presented for Araxá PR; however, all combinations are on the left side of the y-axis and point out the unfeasibility of using this source *in natura*.

The second component (PC2), which explained 21.4% of the variation, separated the combinations mainly in relation to acidification or alkalization (Table A1). Most of the combinations with Bayóvar PR were near or below the x-axis, which showed the alkalization power of this P source, mainly because it contains calcium carbonate. Combinations with the absence of N or when the nitric source was used also favored alkalization of the rhizosphere (Figure A1).

To complement the quantitative description of the patterns presented by the treatments, a correlation analysis was performed involving the factors responsible for the separation of treatments in PCA. For that, all the data points were run through a pairwise correlation analysis (Figure 6A). Out of 55 possible pairs analyzed, 29 resulted in significant correlations ($p \leq 0.05$). Of these, after FDR correction, 25 resulted in significant correlations (Figure 6B). In general, the evaluation parameters of P and Ca were shown to be highly correlated. Describing some more striking correlations, PRR correlated with the total P and Ca content in shoots (P shoots and Ca shoots), total amount of P in roots (P roots) and with the amount of available P in soil (P res). The Ca content in soil (Ca soil) correlated with P res and P content in roots. In fact, these results are expected given that both elements come from the P sources.

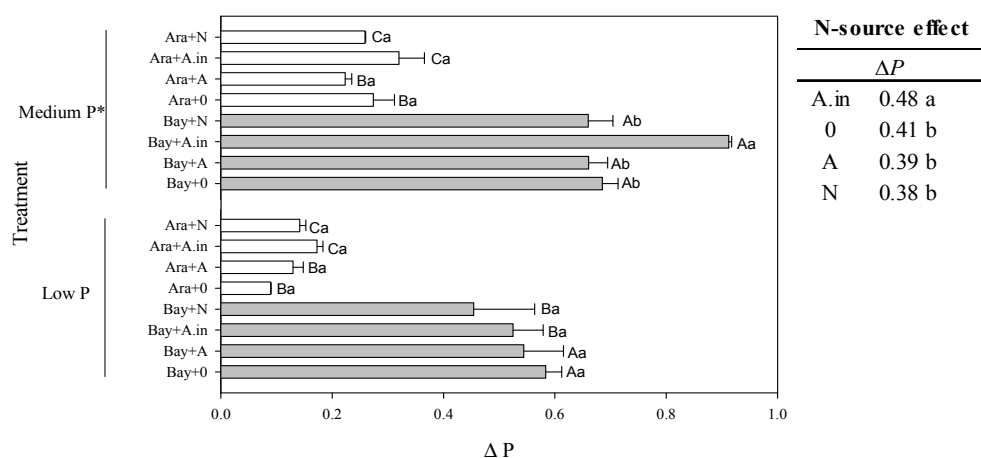


Figure 4. ΔP of millet rhizosphere soil cultivated under 16 different combinations of PR and N sources, at low and medium initial P level. The asterisk indicates that ΔP was greater in plants grown in soil with a medium initial P level (test F). The factorial “P source \times N source” was splitted and shown in the figure. P sources: Ara = Araxá PR, Bay = Bayóvar PR, TSP = triple superphosphate. N sources: 0 = non-application of N, A = ammonium sulfate, A.in = ammonium sulfate with dicyandiamide (DCD) – a nitrification inhibitor agent, N = magnesium nitrate. Uppercase letters compare the mean between the P sources within each N source. Lowercase letters compare the N sources within each P source. Means were compared by the Duncan test at 5% significance. The table on the right shows the effect of N source on ΔP

3.4 Plant Effect and Nitrification

On cultivated soil, the use of Bayóvar PR promoted greater alkalization of the rhizospheric environment, but Araxá PR led to acidification (Figure A1). Nitrogen sources also had significant effects on pH change in the rhizosphere. In this case, for soil with low and medium initial P availability, there was greater alkalization for combinations of Bayóvar PR and nitric source, followed by those with the absence of N. There was acidification when the ammoniacal source was supplied (Figure A1). Millet subjected to the exclusive ammonium supply did not show toxicity symptoms, including no reduction in growth.

To better understand the factors involved in the best use of P by millet, a soil mirror experiment without the presence of plants was also carried out for comparison, aiming to demonstrate the effects of the plants' presence. Ammonium was not detected in soils in the presence of plants, indicating that the plants consumed all the added N source since in the absence of plants there was a coherent quantification of this cation (Figure A2). There were also no significant differences in the amount of nitrate detected; in addition, a small basal amount was found in cultivated soil (Figure A3). Also, for nitrate, there was coherent detection in soil without the presence of plants (Figure A2). These data indicate that there were no large nitrification rates, so that most of the ammonium applied to the soil was maintained. This fact also allows the inference that the pH alterations in soil observed under plant cultivation were related to ammoniacal source uptake and not to the nitrification process.

Without the presence of plants, there were no significant correlations among the variables evaluated in the soil (Figure A4) (except for pH and ΔpH). In these conditions, there was also smaller ΔCa and ΔP in the soil (Figures A5 and A6), evidencing the importance of the plant component in the system to modulate the processes of dissolving less soluble P sources in an oxisol.

4. Discussion

The efficiency of the phosphate sources as fertilizers was shown to be dependent on intrinsic characteristics of the P and N sources and the plant itself. Because a significant area of cultivated tropical soils have medium P availability, due to recurrent P fertilizer application over time, we also tested combinations of P and N sources considering this current scenario. However, there were no differences in PRR on altering the initial P availability of the oxisol, evidencing that highly weathered soils have high P chemisorption capacity (Novais & Smyth, 1999; Parfitt, 1979), keeping a low P concentration in soil solution (Novais & Smyth, 1999). In these conditions, the diffusive flux of P in the soil is low, decreasing its availability for plant growth (Schachtman, Reid, & Ayling, 1998).

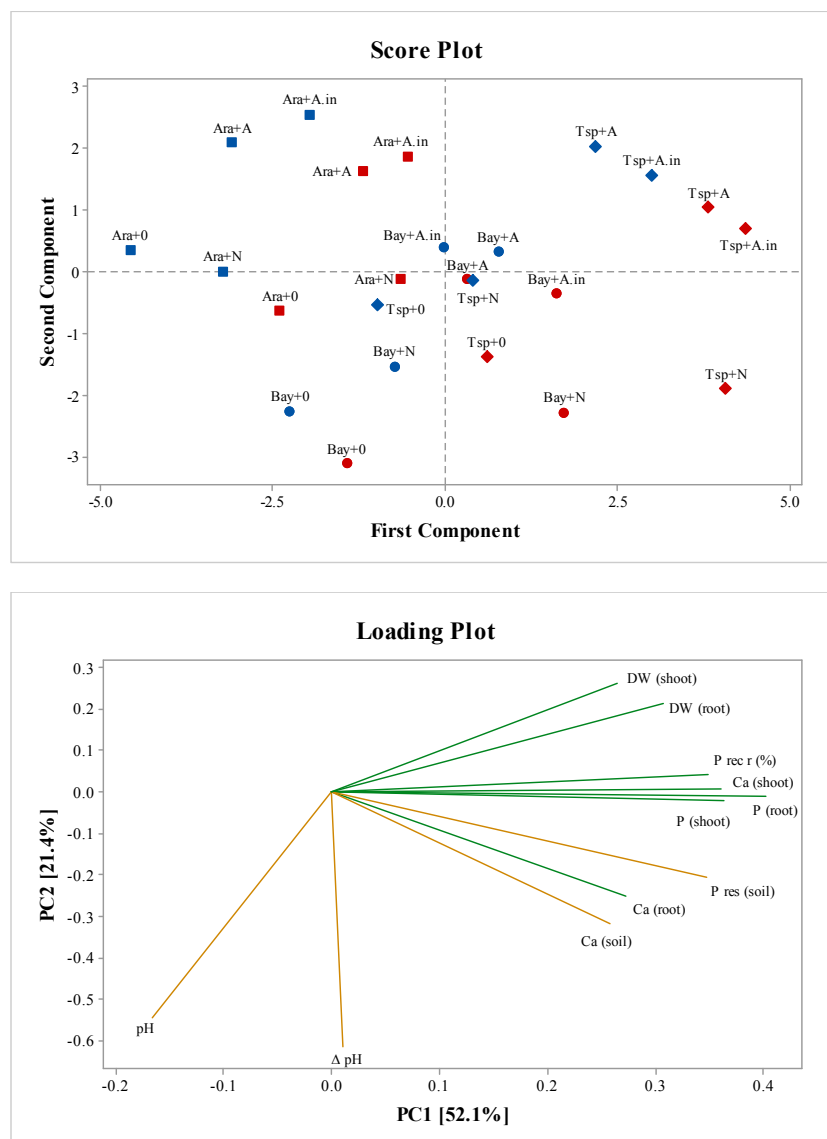


Figure 5. Principal component analysis score plot derived from data of millet plants supplied with different combinations of P and N sources. The blue and red colors indicate plants grown under low and medium P initial availability in an oxisol, respectively. The P fertilizers are presented by different shapes: TSP (diamond), Bayóvar PR (circle) and Araxá PR (square). The combinations of P and N sources are indicated in the graph. In the loading plot, the direction and length of the lines are directly proportional to their importance in separating groups. PC1, principal component 1, explaining 52.1% of the variance; PC2, principal component 2, explaining 21.4% of the variance. Abbreviations: dry weight of root (DW (root)), dry weight of shoot (DW (shoot)), available P by the anionic resin extractor (P res), P recovery rate (P rec r (%)) and difference in soil pH before and after the experiment (Δ pH)

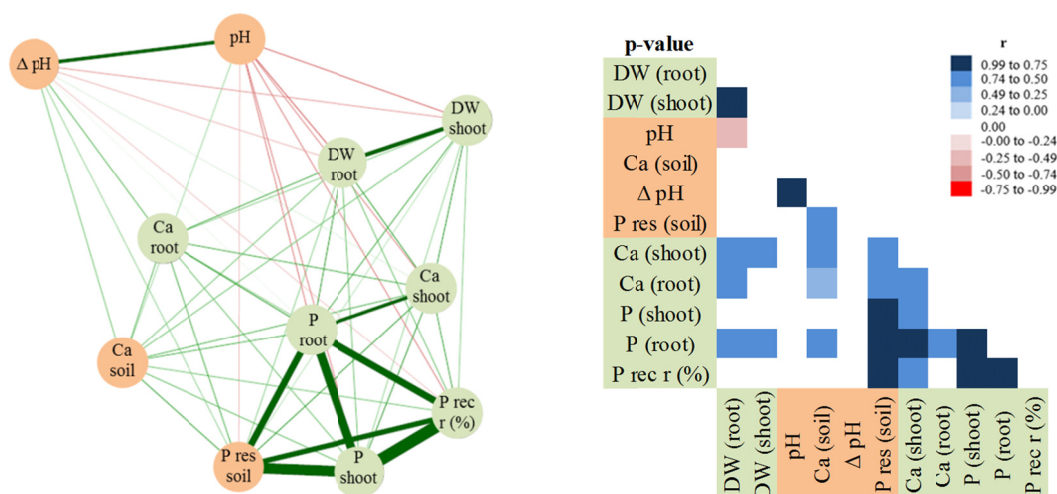


Figure 6. (A) Correlation network between variables derived from data of millet plants supplied with different combinations of P and N sources. Red and green lines represent negative and positive correlations, respectively. Line width is proportional to the strength of the correlation. (B) Heat map of correlations between soil and plant parameters of 24 combinations of P (Bayóvar PR, Araxá PR and TSP) and N (N-NH_4^+ and N-NO_3^-) sources under different initial P levels (P-low and P-medium). Each square represents significant correlation between the parameters heading the column with the parameters heading the row. Correlation coefficients and significances were calculated by Pearson correlations ($p < 0.05$). Blue and red squares represent positive and negative correlations, respectively; in addition, the correlation data were corrected by base FDR (Benjamini & Hochberg, 1995)

Some studies have shown positive results when P is supplied by combining soluble sources with PR, for development of root systems, which promotes greater agronomic efficiency in PR use (Chien, Prochnow, Tu, & Snyder, 2011; Prochnow, Chien, Carmona, & Henao, 2004). In our development, there was better P availability in the soil when the initial P availability was at a medium level, but it did not lead to a greater PRR by plants. In addition, we can observe that PRR was strongly related to the solubility of P sources. TSP promoted higher PRRs, followed by Bayóvar PR and Araxá PR. PR of sedimentary origin (Bayóvar PR) is more reactive than that from igneous deposit (Araxá PR) because it contains more poorly crystalline apatites (Santos et al., 2016), leading it to release P into soil solution at higher rates. In addition, in the PCA approach, PC1 clearly reflected the contrasting effect of P-source solubility on the factors evaluated (Figure 5).

The combination of a soluble phosphate fertilizer (TSP) with N-NH_4^+ allowed a higher PRR than the combination with N-NO_3^- , regardless of the initial P availability. Two factors possibly explain this response: greater P uptake and greater P diffusion in the presence of ammonium. Greater P uptake in the presence of ammonium has already been observed in several works, especially considering calcareous and alkaline soils (Jing et al., 2010; Ortas, Harris, & Rowell, 1996; Wang, Guppy, Watson, Sale, & Tang, 2011). An improvement of the PRR for ammoniacal source use was recently reported by Valadares et al. (2017), but only when soluble phosphate fertilizers were used.

Using nutrient solution, it was verified that P uptake can be stimulated in rice plants by supplying them with NH_4^+ because of the greater activity of H^+ -ATPase in the plasma membrane of the root cells (Zeng et al., 2012). In addition, when verifying that P accumulation increased with decreasing NO_3^- supply, it has been proposed that there is an antagonistic genetic interaction of P with nitrate but not with ammonium in *Arabidopsis* (Kant et al., 2011). On the other hand, another factor that may have contributed to the better performance of the P-soluble source is the greater diffusion of orthophosphate in water when ammonium ions are present (Villani et al., 1998). This effect may have been insignificant for PRs since the solubilization rate may not allow P availability coincident with N uptake.

The small values of N-NH_4^+ and N-NO_3^- in soil under millet showed the consumption of a large part of the applied N source. Millet has shown to be tolerant to the use of N-NH_4^+ . Not all plant species are tolerant to the exclusive ammonium supply and its effects on plant and soil (Gweyi-Onyango, Neumann, & Romheld, 2005; Kronzucker et al., 1997; Sarasketa, González-Moro, González-Murua, & Marino, 2014). Possibly, the tolerance of millet to these conditions is due to its tropical origin and its having gone through few processes of crop

breeding (Bloom, Jackson, & Smart, 1993; Kronzucker, Britto, Davenport, & Tester, 2001). In this context, we can highlight root system maintenance, considering the importance of this factor in the efficiency of P acquisition (Lambers, Shane, Cramer, Pearse, & Veneklaas, 2006). It is known that ammonium modifies the root system by inhibiting root elongation, stimulating lateral root branching or swelling root hairs (Liu & von Wirén, 2017).

In consequence of the N-NH₄⁺ or N-NO₃⁻ supply, the rhizosphere pH was altered in maintaining cellular electrical neutrality (Dakora & Phillips, 2002; Gahoonia, Claassen, & Jungk, 1992; Haynes, 1990; Philippe Hinsinger, Plassard, Tang, & Jaillard, 2003). Greater buffering of pH and exchangeable Ca²⁺ was observed when Bayóvar PR was supplied because it is from a marine sedimentary deposit, where its occurrence is associated with calcium carbonate ores. In PCA analysis, PC2 explained 21.4% of the variance, and the pH alteration caused by the combinations was the major component (Figure 5).

We used non-plant rhizotubes to verify if the nitrification process could contribute to the pH change in the rhizosphere. With this, we verified that nitrification did not significantly affect the rhizospheric acidity, supposedly due to the low rates achieved. Other studies also report a low nitrification rate in soil (Khalil, Boeckx, Rosenani, & Cleemput, 2001; Zhao, Cai, & Xu, 2007).

In the absence of plants, the factors evaluated had much lower positive correlations and fewer soil alterations (Figure A4), and poor solubilization of the PRs. These results demonstrate the importance of the presence of plants in the soil in promoting conditions for solubilizing PRs or increasing the plant P availability, including their effects such as Ca and P drainage, rhizosphere acidification and stimulating the biological activity that can contribute to P solubilization and cycling.

5. Conclusion

This study shows that there is no effect of previous medium P availability on PRR regardless of phosphorus source used in oxisols. In addition, it provides evidence that the presence of cover plants on oxisols is crucial for increasing the efficiency of phosphorus fertilizers and their plant availability over time. Improvement of PRR when an ammoniacal source is used is observed when soluble phosphate fertilizers are used. Millet is highlighted as a useful crop for promoting solubilization of PRs, with the advantage of being a high biomass-yielding plant promoting efficient cover of the soil surface.

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Appendix A

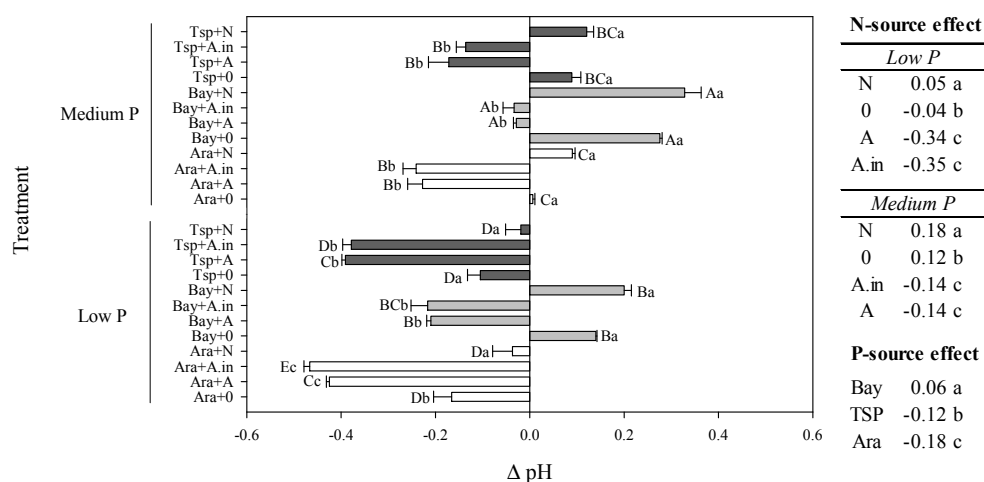


Figure A1. Δ pH of millet rhizosphere soil cultivated under 24 different combinations of P and N sources, at low and medium initial P level. The factorial “P source \times N source” was splitted and shown in the figure. P sources: Ara = Araxá PR, Bay = Bayóvar PR, TSP = triple superphosphate. N sources: 0 = non-application of N, A = ammonium sulfate, A.in = ammonium sulfate with dicyandiamide (DCD)—a nitrification inhibitor agent, N = magnesium nitrate. Lowercase letters compare the N sources within the same P source. Means were compared by the Duncan test at 5% significance. The table on the right shows the interaction of “N source \times P initial level” and the effect of the P source on Δ pH

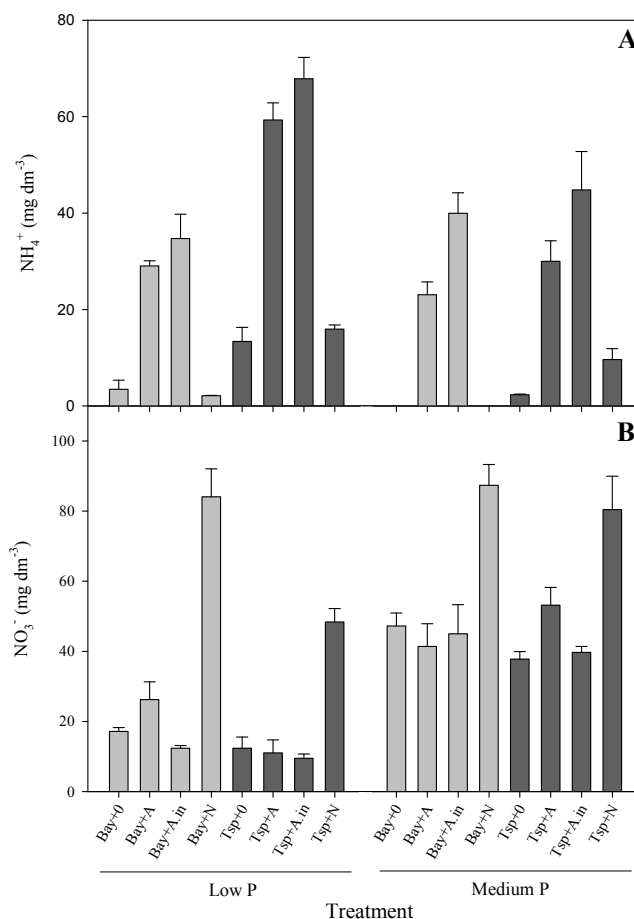


Figure A2. NH_4^+ (A) and NO_3^- (B) levels in oxisol treated with 16 different combinations of P (Bayóvar PR and TSP) and N sources, at low and medium initial P level

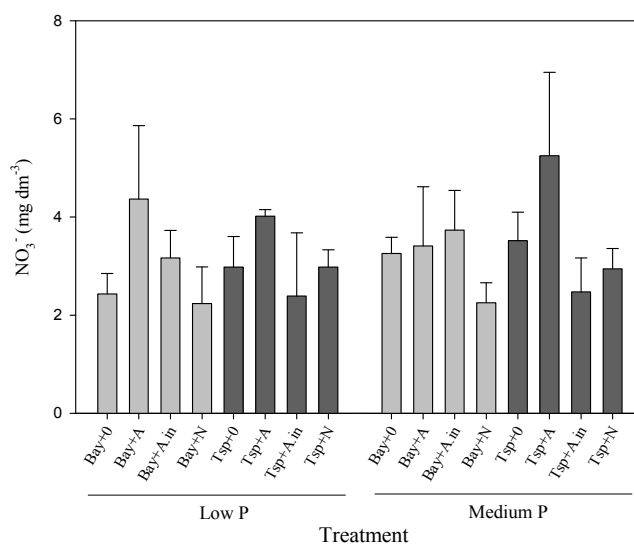


Figure A3. NO_3^- in millet rhizosphere soil cultivated under 16 different combinations of P (Bayóvar PR and TSP) and N sources, at low and medium initial P level. NH_4^+ was not detectable

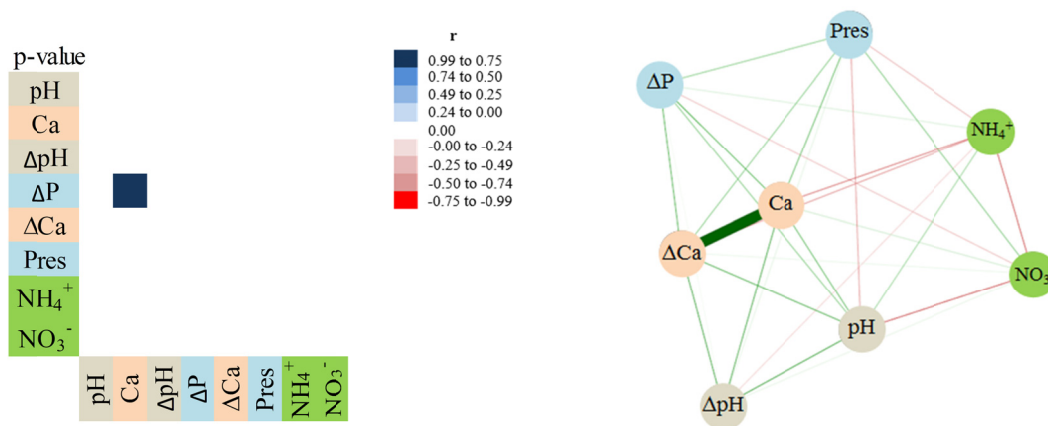


Figure A4. (A) Heat map of correlations between soil parameters of 24 combinations of P (Bayóvar PR, Araxá PR and TSP) and N (NH_4^+ and NO_3^- sources) sources under different initial P level (P-low and P-medium) in the absence of plants. Each square represents significant correlation between the parameters heading the column with the parameters heading the row. Correlation coefficients and significances were calculated by Pearson correlations ($p < 0.05$). Blue and red squares represent positive and negative correlations, respectively; in addition, the correlation data were corrected by base FDR (Benjamini and Hochberg, 1995). (B) Correlation network of the same parameters evaluated. Red and green lines represent negative and positive correlations, respectively. Line width is proportional to the strength of the correlation

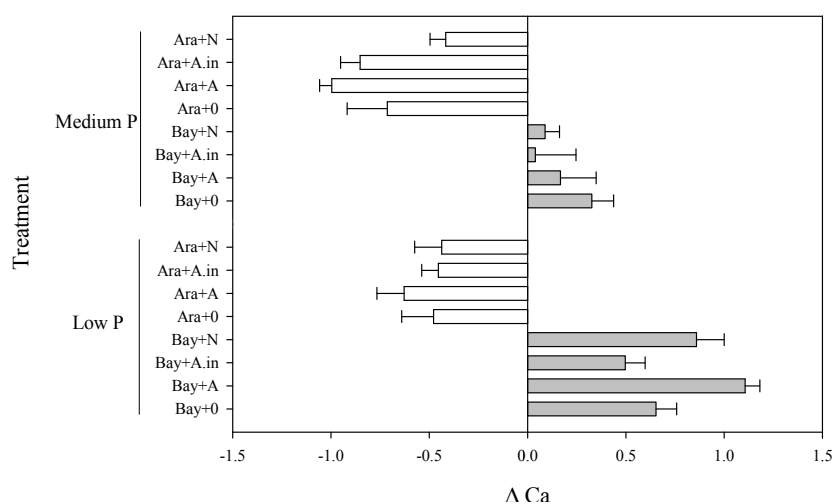


Figure A5. ΔCa of millet rhizosphere soil cultivated under 16 different combinations of PR and N sources, at low and medium P level in the absence of plants. P sources: Ara = Araxá PR, Bay = Bayóvar PR, TSP = triple superphosphate. N sources: 0 = non-application of N, A = ammonium sulfate, A.in = ammonium sulfate with dicyandiamide (DCD)—a nitrification inhibitor agent, N = magnesium nitrate

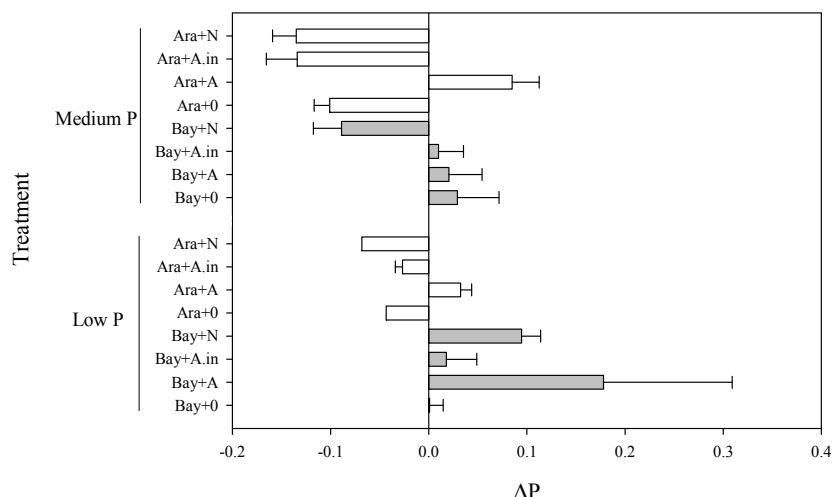


Figure A6. ΔP of millet rhizosphere soil cultivated under 16 different combinations of PR and N sources, at low and medium initial P level in the absence of plants. P sources: Ara = Araxá PR, Bay = Bayóvar PR, TSP = triple superphosphate. N sources: 0 = non-application of N, A = ammonium sulfate, A.in = ammonium sulfate with dicyandiamide (DCD)—a nitrification inhibitor agent, N = magnesium nitrate

Table A1. Loading factors of the principal components that best separate the data. PC1 and PC2 model 52.1% and 21.4% of the data matrix variance, respectively. The variables were ordered from the highest to the lowest values

PC1		PC2	
Variable	Loading factor	Variable	Loading factor
P (root)	0.397	ΔpH	0.611
Recovery rate (%)	0.375	pH	0.549
P (shoot)	0.36	Ca (soil)	0.305
Ca (shoot)	0.357	Ca (root)	0.234
P res (soil)	0.349	P res (soil)	0.202
DW (root)	0.303	P (shoot)	0.021
Ca (root)	0.274	Recovery rate (%)	0.017
Ca (soil)	0.256	P (root)	-0.002
DW (shoot)	0.255	Ca (shoot)	-0.024
ΔpH	0.023	DW (root)	-0.234
pH	-0.161	DW (shoot)	-0.283

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