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Interaction between Phosphorus and Nitrogen in Organomineral Fertilizer

Marco André Grohskopf ^a, Juliano Corulli Corrêa^b, Dirceu Maximino Fernandes^a, Paulo César Teixeira^c, Caio Vilela Cruz^a, and Sandra Camile Almeida Mota^b

^aDepartment of Soil Science, São Paulo State University “Júlio de Mesquita Filho”, Botucatu, Brazil; ^bEmbrapa Swine and poultry, Brazilian Agricultural Research Corporation (Embrapa), Concórdia, Brazil; ^cEmbrapa Soils, Brazilian Agricultural Research Corporation (Embrapa), Rio de Janeiro, Brazil

ABSTRACT

The aim of this research is to assess the effect of the interaction between phosphorus and nitrogen (P × N) in organomineral fertilizer in the nutrition of plants cultivated in a Typic Hapludox. It was used a completely randomized design in a 5 × 5 factorial scheme with four replications. The treatments corresponded to the equivalent of five doses of P (0, 20, 40, 60, and 80 kg ha⁻¹ of P) in interaction with five doses of N (0, 50, 100, 150, and 200 kg ha⁻¹ of N). Three sequential crops of millet were carried out with 40 days of growth each, whose aerial part of the plants was harvested in order to determine the dry biomass and the contents of N and P. After each crop, soil samples were collected from the pot for determination of the forms of P and N. It may be inferred that simultaneous application of N and P into the fertilization does not promote significant changes in the dynamics of N in the soil, but the effect of the interaction is changed over the crop time in the plant. In soil the application of organomineral fertilizers did not show effect of the interaction between P × N on the forms of organic N, inorganic and organic P, except for a difference in the available P, which is the dose of 20P × 200N kg ha⁻¹ with greater efficiency. In the plant, there was interaction between P × N in organomineral fertilizer on the dry biomass of the aerial part of millet from the dose of 20P × 100N, focusing on the dose of 50P × 150N kg ha⁻¹, while the accumulation of P and N starts from the dose of 40P × 100N and 40P × 50N kg ha⁻¹ with an increase (synergy) in both nutrients up to the dose of 80P × 200N kg ha⁻¹.

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Introduction

The development of new fertilizers is strategic for agribusiness, arising from the high demand for imports because 75% of fertilizers consumed in Brazil are imported (ANDA, 2016). Among new technologies, the organomineral fertilizers – which are prepared from raw materials available in large scale, such as the wastes of poultry litter, in a mix with mineral fertilizers – allow the production of formulations adapted to the nutritional demands of crops for different soil classes and reuse of such waste at greater distances from production concentration areas (Corrêa et al. 2016).

Brazilian soils are primarily oxidic and have a high level of weathering with low natural fertility (Cunha et al. 2014), which are features demanding high requirements of application of fertilizers, mainly for Nitrogen (N) and phosphorus (P). The concentration and forms of these nutrients in the soil are determining factors for the yield of agricultural systems (Fageria and Baligar 2014; Lacerda et al. 2015).

CONTACT Marco André Grohskopf  marcogrohskopf@gmail.com  Department of Soil Science, São Paulo State University “Júlio de Mesquita Filho”, Botucatu, São Paulo, Brazil

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In these soils, the efficiency of phosphate fertilizers is related to the adsorption and precipitation reactions that control the destination of P (Montalvo, Degryse, and McLaughlin 2015), especially its adsorption due to the high bond energy between the phosphate anion with functional groups on mineral surface (Bolan et al. 2013; Shuai and Zinati 2009). The N efficiency of the fertilizers is related to many processes, such as desorption, adsorption, leaching, volatilization, nitrification, denitrification, immobilization, and mineralization (Zhang et al. 2011).

Most scientific studies evaluate the use of these nutrients separately in fertilization, whose effect of the interaction between phosphorus and nitrogen (P × N) and the mechanisms involved in this process are insufficiently studied. In crop fertilization, there is a frequently concomitant supply of P and N together in the fertilizer at the time of the localized application or in the soil total area. Thus, the efficiency of organomineral fertilizers can also be associated to the interactions of these elements in the formulation, interfering with the dynamics of these nutrients in the soil and in plant nutrition.

In the plant, there are many interaction points depending on P and N; however, the mechanisms of interaction between these elements are still insufficiently clarified (Agren, Wetterstedt, and Billberger 2012). Plants normally do not respond to N fertilization due to soil P deficiency conditions (Setiyono et al. 2010). At adequate or high levels of P in the plant, there is an increase in N uptake and accumulation, while increasing the N dose increases the P uptake (Agren, Wetterstedt, and Billberger 2012; Duncan et al. 2018a). Therefore, N and P interact synergistically. Both nutrients at adequate levels promote an increase in vegetable production in relation to the production obtained with the application of each nutrient individually (Fageria and Baligar 2014; Sattari et al. 2014).

The concentration of N and P varies in the soil over time, which can create interactions between the nutrients and result from the synergistic or antagonistic combination. This could directly or indirectly affect the response of crops (Duncan et al. 2018b; Groot et al. 2003). Therefore, the concomitant supply of these nutrients in soil results in changes of chemical, physical, and biological features (Chen et al. 2017; Makhani and Isaac 2014; Yokoyama, Imai, and Kitayama 2017) that can modify that dynamics of these elements.

The objective of this work was to evaluate the effect of the interaction between phosphorus and nitrogen, interacting in five concentrations of each nutrient in organomineral fertilizer produced from poultry litter, on the growth of millet plant, also on organic phosphorus and nitrogen in a Typic Hapludox.

Material and methods

Experimental characterization

The experiment was conducted in a greenhouse under controlled conditions at São Paulo State University “Júlio de Mesquita Filho,” Botucatu Campus – SP. The samples used were collected from a soil classified as clayed *Latossolo Vermelho Distroférrico* (clayed Typic Hapludox) (Santos et al. 2013). Soil samples were collected in the 0–0.2 m depth layer in Botucatu, SP, in an area of native vegetation, without fertilizer and corrective application. Subsequently, the collected samples were dried and sieved with a 4mm mesh, removing roots, straws, and lumps. The soil chemical and physical characterization was performed according to Van Raij et al. (2001) and is presented in Table 1.

Table 1. Initial features of the layer of 0.0 to 0.2 m of typic hapludox (Oxisol) used in the experiment. Botucatu, SP, 2014.

Soil	pH	PCZ ⁽¹⁾	V	Al	H+ Al	Ca	Mg	K	CTC	P _{Mehlich} ⁽²⁾	Clay	MOS	SD
	CaCl ₂		%		—	mmol _c dm ⁻³	—			mg dm ⁻³	g kg ⁻¹	g dm ⁻³	g cm ⁻³
Oxisol	4.0	4.4	13	17	89	9.0	3.0	0.7	102	5	474	17.0	1.2

Key – ⁽¹⁾ Point of Zero Charge; MOS = Soil Organic Matter; SD = Soil Density; V: Base saturation; Al: interchangeable aluminum; Ca: interchangeable calcium; Mg: interchangeable magnesium; ⁽²⁾ P: extractable phosphorus, method of Mehlich; K: extractable potassium.

The soil parent material used in the experiment originated from basalt rock. It is characterized as oxidic soil with the prevalence of crystalline forms of iron oxides (goethite and hematite). In the clay fraction, kaolinite is predominant, followed by 2:1 clay minerals with polymers of aluminum hydroxide among layers. In lower proportions, there are gibbsite and quartz (Carvalho, Espíndola, and Paccola 1983).

The remaining P value (Rem-P) was 13.6 mg kg^{-1} and was determined according to Alvarez V et al. (2000). The determination of Rem-P was performed by shaking in a horizontal shaker for five minutes, 5 g of a soil sample in 50 mL of CaCl_2 solution (0.01 mol L^{-1}) containing 60 mg L^{-1} of P. After 16 h of stirring, the P concentration was determined in the equilibrium solution by colorimetry.

The maximum adsorption capacity of P (C_{MAP}) by the Langmuir isotherm was 729.6 mg kg^{-1} (Figure 1) and was determined according to procedures described by Graetz and Nair (2000). In the procedure, 2.5 g of soil were placed in CaCl_2 solution (0.01 mol L^{-1}) with different concentrations of P up to 180 mg L^{-1} of the KH_2PO_4 solution (1000 mg L^{-1}), calculated according to the remaining P (Rem-P). Thereafter, centrifugation was performed at 3000 rpm for 15 min and the P content in the supernatant was determined by colorimetry using the ascorbic acid method (Braga and Defelipo 1974).

Experimental design

The experiment was conducted with a completely randomized design in 5×5 factorial scheme with four replications. The treatments were composed of five doses of phosphorus (P) (0, 20, 40, 60, and 80 kg ha^{-1} of P – elemental form) in interaction with five doses of nitrogen (N) (0, 50, 100, 150, and 200 kg^{-1} of N) in organomineral fertilizer. Different formulations of organomineral fertilizer were produced to form the doses of interaction between P and N, whose formulas are represented by the values of $\text{P}_2\text{O}_5 - \text{N}$: 0–0 (control); 0–15; 6–0; 6–1.5; 6–2; 6–3; 6–4; 6–4.5; 6–5.5; 6–6; 6–7.5; 6–8; 6–8.5; 6–11.5; 6–12; 6–17; and 6–22 (Table 2). The experimental units consisted of polyethylene pots containing 10.0 kg of soil dry mass.

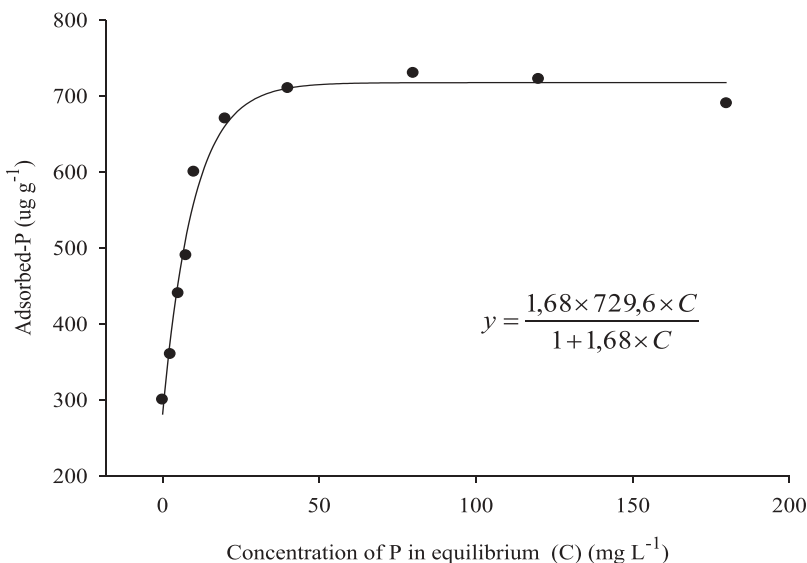


Figure 1. Maximum adsorption capacity of phosphate of the Typic hapludox in natural conditions before installing the experiment, adjusted by means of the adsorption curve by the Langmuir Isotherm.

Fertilizers and soil preparation

For the production of organomineral fertilizer (OMF) formulations, the organic material consisted of poultry litter, collected after six consecutive lots of broiler chickens with 42 days each and showed 20, 20, and 18 g kg⁻¹ of N, P, and K, respectively, in elemental form (dry basis composition). For OMF formulation in complex form, the poultry litter was present between 50 to 85% of the composition (according doses formulated), and the content of N and P in this organic waste was considered.

As a mineral source, urea (CO(NH₂)₂) (45% of N) and triple superphosphate (Ca(H₂PO₄)₂·2H₂O) (46% P₂O₅) were used in powder form. The proportions of mineral sources to achieve the pre-established formulas are shown in Table 2, to formulate the interactions between P and N described for each treatment. For granulation of the OMF, the poultry litter was shredded and sieved in a sieve with a 1.0mm mesh.

The mixture of materials (organic and mineral) was pre-homogenized with defined amounts of each material for each formulation to be tested. It was subsequently placed in a disc granulator by adding 1% of bentonite as a hardness adjuvant and 0.5% of neutral calcium silicate solution as a binding agent to support the granulation of the fertilizer. After the granulation process, the OMF was sifted in a 4 and 1 mm mesh sieve, separating the material retained between the two sieves, which was dried in a forced circulation system at 65°C for 24 h or until reaching a constant weight.

The soil acidity was corrected to increase the base saturation to 70% using dolomitic limestone (total neutralizing power = 100%) in the 2:1 CaCO₃ and MgCO₃ ratio, incubating for 20 days. After this period, except for elements under test (N and P), the doses of 160; 23; 1.3; 0.9; and 1.1 mg kg⁻¹ potassium, sulfur, copper, zinc and boron were applied using KCl, CaSO₄, CuSO₄, ZnSO₄ and Borax (Na₂B₄O₇·10H₂O) in the soil to provide high-level availability to the plants. Then, the fertilizer formulations for the interactions between P and N were incorporated into the soil in the 0.04m depth furrow in the center of each pot, simulating the localized application aligned and apart from the seeds.

Table 2. Scheme of the procedures due to increasing doses of P and N that constituted the organo-mineral fertilizer and experimental units.

Dose of P	Dose of N	Formulation	Amount	Dose g/vaso
Kg ha ⁻¹	Kg ha ⁻¹	P ₂ O ₅ -N	Kg ha ⁻¹	10 kg of soil
0	0	0-0	0	0
20	0	00-15	310	1.55
40	0	00-15	620	3.10
60	0	00-15	930	4.65
80	0	00-15	1240	6.20
0	50	06-00	833	4.16
0	100	06-00	1667	8.33
0	150	06-00	2500	12.49
0	200	06-00	3333	16.66
20	50	06-06	833	4.16
20	100	06-03	1667	8.33
20	150	06-02	2500	12.49
20	200	06-1.5	3333	16.66
40	50	06-11	833	4.16
40	100	06-06	1667	8.33
40	150	06-04	2500	12.49
40	200	06-03	3333	16.66
60	50	06-17	833	4.16
60	100	06-8.5	1667	8.33
60	150	06-06	2500	12.49
60	200	06-4.5	3333	16.66
80	50	06-22.5	833	4.16
80	100	06-11	1667	8.33
80	150	06-7.5	2500	12.49
80	200	06-5.5	3333	16.66

Plant experiment

Three sequential millet crops (*Penisetum glaucum* Leeke) were carried out with 40 days of growth each, counted from the plant emergence. Millet crop was carried out with eight plants per pot remained under intermittent irrigation of water, aiming to maintain the soil moisture close to 80% of the field capacity.

At the end of each crop, the dry shoot biomass production was determined by cutting close to the soil surface. The vegetal material was dried in a forced air circulation system at 60°C for 72 h or until reaching constant weight, and weighed. Subsequently, the material was ground in a 1.0-mm sieve mill to determine the total N and P contents, according to Van Raij et al. (2001). The accumulated amount of each nutrient (mg/pot) was estimated by its concentration in the shoot (g kg^{-1}) multiplied by the dry mass (g/pot).

In each crop, after 40 days of the plant growth, the soil was collected in order to carry out chemical analyses. The pot soil was collected by removing a sample perpendicular to the furrow of fertilizer deposition with 0.05m wide and 0.20 m depth. Subsequently, the samples were homogenized and identified.

After collection, the soil was dried in a forced air circulation system at 65°C and then ground and sieved with 2.0-mm mesh for chemical determinations of the N and P forms.

Measurements and analyses

In the soil, the available P content was determined by anion-exchange resin, as described by Van Raij et al. (2001). Total P was determined by digestion ($\text{H}_2\text{SO}_4 + \text{H}_2\text{O}_2 + \text{MgCl}_2$), according to Brookes and Powlson (1981), and total organic P as described by Bowman (1989), whose determination of P of extracts were according to the methodology of Murphy and Riley (1962).

The fractioning of N organic forms was based on extraction and quantification of nitrogen compounds released from acid hydrolysis and based on the degradation technique of proteins, according to Yonebayashi and Hattori (1980), as procedures described in Grohskopf et al. (2015).

Statistical analysis

The results were submitted to analysis of homogeneity, variance, and normality. After compliance with parametric statistical assumptions, the analysis of variance was carried out by the F test, considering the completely randomized experimental model to evaluate the treatment effect. In case of significant differences, the treatment effect was compared by Tukey test ($P \leq 0.05$).

Results and discussion

Fertilization by organomineral fertilizers at different rates of interaction between $P \times N$ did not promote changes in total, organic, and inorganic P contents in the soil (Table 3). The values of significance (p -value) when comparing among three crops for P forms were 0.42, 0.22, and 0.26 for the forms of total P, organic P, and inorganic P, respectively. It should be remembered that the soil was collected after the growth period of the plants in each crop, whose values are represented by an average of three samples.

Among the forms of P assessed, the organic P represented the lower proportions in an average of 22% of the total P in the soil (Table 3), and the larger part of P are inorganic forms. The fact that the fertilizer does not alter the forms of P in the soil can be explained because it does not present modification in a short period, like in experiment in pots, since there were not high amounts of mineral or organic P by organomineral fertilizers.

There was effect for the available P contents due to the fertilization with organomineral at different doses of interaction between $P \times N$ (Table 4). After the first and second crops of millet,

Table 3. Content of total phosphorus (P), total organic P and inorganic P in the soil after sequential crops of millet in response to application of organomineral fertilizer in different doses of interaction between phosphorus and nitrogen (P × N).

Dose of P (kg ha ⁻¹)	Dose of N (kg ha ⁻¹)				
	0	50	100	150	200
Total P (mg kg⁻¹) (*ns)					
0	1486 ± 22 ^a	1479 ± 26	1496 ± 13	1491 ± 32	1488 ± 26
20	1491 ± 32	1483 ± 49	1506 ± 31	1496 ± 30	1519 ± 37
40	1488 ± 41	1496 ± 28	1481 ± 39	1503 ± 36	1536 ± 52
60	1501 ± 39	1480 ± 24	1488 ± 40	1529 ± 28	1527 ± 27
80	1509 ± 32	1497 ± 33	1499 ± 37	1507 ± 43	1531 ± 28
Total organic P (mg kg⁻¹) (*ns)					
0	297 ± 8	301 ± 14	333 ± 15	299 ± 14	313 ± 12
20	327 ± 10	299 ± 11	325 ± 13	306 ± 16	308 ± 11
40	312 ± 11	304 ± 16	322 ± 19	314 ± 21	321 ± 22
60	300 ± 9	318 ± 13	309 ± 9	320 ± 17	327 ± 15
80	317 ± 10	311 ± 10	340 ± 18	314 ± 16	306 ± 10
*Inorganic P (mg kg⁻¹) (*ns)					
0	1189 ± 39	1178 ± 41	1163 ± 42	1192 ± 39	1175 ± 44
20	1164 ± 38	1184 ± 40	1181 ± 40	1190 ± 37	1211 ± 46
40	1176 ± 39	1192 ± 40	1159 ± 46	1189 ± 41	1215 ± 37
60	1201 ± 50	1162 ± 34	1179 ± 48	1209 ± 42	1200 ± 41
80	1192 ± 40	1186 ± 37	1159 ± 49	1193 ± 40	1225 ± 41

^aAverages of the three crops. * Values calculated by the difference between the total P and organic P. (*ns) Not significant from Tukey test (p ≤ 0.05).

there were interactions for contents of available P from 40P × 50N and 20P × 50N kg ha⁻¹. While in the third crop, the interactions remain, but at doses higher than N from 20P × 100N kg ha⁻¹. When there was no application of N, the increase of P always raised the content of P in the three crops.

The low response of the available P can be explained by its strong interaction with the mineral phase of this soil and specific adsorption, forming high-level stability complexes (Shen et al. 2014; Shuai and Zinati 2009), and making P less available. As the saturation of the most avid locations in the soil occurs by adsorption process, P starts to form less energetic bonds in monodentate complexes with higher lability and availability (Barrow 2015), explaining the increase of available P in the highest doses of organomineral fertilizers.

Table 4. Content of phosphorus (P) available of the soil after sequential crops of millet in response to application of organomineral fertilizer in different doses of interaction between phosphorus and nitrogen (P × N).

Dose of P (kg ha ⁻¹)	Dose of N (kg ha ⁻¹)				
	0	50	100	150	200
Crop 1					
0	4 ± 0.5 Aa	4 ± 0.4 Ca	4 ± 0.4 Ba	4 ± 0.4 Ca	4 ± 0.5 Ba
20	7 ± 1.5 Ab	8 ± 0.6 Bb	9 ± 1.1 Ab	12 ± 1.4 Ab	19 ± 1.9 Aa
40	6 ± 0.9 Ac	19 ± 3.2 Aa	8 ± 1.7 Ac	11 ± 1.2 Ab	21 ± 1.4 Aa
60	6 ± 0.7 Ab	19 ± 3.1 Aa	7 ± 0.9 Ab	8 ± 0.4 Ab	21 ± 1.6 Aa
80	7 ± 1.5 Ab	15 ± 3.2 Aa	8 ± 1.7 Ab	8 ± 0.6 Ab	18 ± 5.1 Aa
Crop 2					
0	4 ± 0.3 Ca	3 ± 0.3 Ba	3 ± 0.4 Ba	4 ± 0.3 Ba	4 ± 0.2 Ba
20	5 ± 0.6 Cc	7 ± 1.6 Ac	10 ± 2.4 Ab	11 ± 1.4 Ab	16 ± 1.1 Aa
40	11 ± 2.0 Bb	5 ± 0.9 Ac	6 ± 0.6 Ac	9 ± 0.9 Ab	17 ± 1.5 Aa
60	12 ± 1.2 Bb	5 ± 1.1 Ac	7 ± 0.8 Ac	12 ± 2.5 Ab	20 ± 1.5 Aa
80	20 ± 2.3 Aa	6 ± 1.1 Ac	10 ± 2.5 Ab	10 ± 1.2 Ab	16 ± 3.6 Aa
Crop 3					
0	4 ± 0.3 Ba	6 ± 0.4 Aa	6 ± 0.2 Ca	4 ± 0.3 Ba	4 ± 0.3 Ba
20	6 ± 0.4 Bc	7 ± 0.5 Ac	21 ± 1.3 Ab	19 ± 1.2 Ab	29 ± 0.7 Aa
40	20 ± 1.4 Ab	6 ± 0.5 Ac	21 ± 1.5 Ab	23 ± 1.5 Ab	28 ± 2.0 Aa
60	21 ± 2.1 Ab	6 ± 0.8 Ad	14 ± 1.4 Bc	19 ± 1.1 Ab	30 ± 2.0 Aa
80	24 ± 2.5 Ab	7 ± 0.8 Ad	16 ± 1.3 Bc	19 ± 1.1 Ac	29 ± 1.7 Aa

Averages connected by distinct letters (lowercase letters in horizontal and uppercase in vertical) differ from Tukey test (p ≤ 0.05).

For the total N and N hydrolyzed levels, there was no change in $P \times N$ interaction in response to the application of organomineral fertilizer in the three crops (Table 5). Hydrolyzed N values correspond on average to 84% of soil total N. Using this same procedure of analysis, Grohskopf et al. (2015) found a proportion of approximately 78% of hydrolysis in the 0 to 0.20 m depth layer in a Typic Hapludox (Oxisol) under no-tillage conditions.

The fact that all organic N in the soil is not extracted by hydrolysis can be explained by the existence of forms in a higher level of stability, by the formation of clay-metal-humus complexes in the soil, by the interaction of the organic molecules, multivalent cations and minerals of clay fraction (Grohskopf et al. 2015), especially Fe and Al oxides predominant in the soil studied. These forms of higher recalcitrance, preserved by mechanisms of physical and chemical protection, and stabilized in the soil microaggregates, limit the action of acid hydrolysis by the method used in this study. This part of non-soluble N in the method can be considered a non-labile fraction of high complexity and stability in the soil.

For the organic N fractions in the forms of $N-NH_4^+$, hexosamine-N, amide-N, α -amino N and unidentified-N, there was no interaction effect between $P \times N$ by the organomineral fertilizers on the

Table 5. Content of total nitrogen (N) and hydrolyzed⁽¹⁾ N of soil after sequential crops of millet in response to application of organomineral fertilizer in different doses of interaction between phosphorus and nitrogen ($P \times N$).

Dose of P (kg ha ⁻¹)	Dose of N (kg ha ⁻¹)				
	0	50	100	150	200
Crop 1					
Total N (mg kg ⁻¹) (*ns)					
0	605 ± 2.9	569 ± 18.5	640 ± 38.9	605 ± 18.5	605 ± 2.9
20	602 ± 2.5	655 ± 14.4	605 ± 14.4	600 ± 8.2	582 ± 20.6
40	585 ± 10.4	578 ± 2.5	605 ± 14.4	615 ± 10.4	610 ± 4.1
60	608 ± 32.8	578 ± 10.0	620 ± 20.4	590 ± 20.4	620 ± 4.1
80	588 ± 2.5	600 ± 10.0	582 ± 2.5	682 ± 4.8	655 ± 14.4
Hydrolyzed N (mg kg ⁻¹) (*ns)					
0	514 ± 2.5	488 ± 15.7	544 ± 33.1	514 ± 15.7	514 ± 2.5
20	512 ± 2.1	557 ± 12.3	514 ± 12.3	510 ± 6.9	505 ± 17.5
40	497 ± 8.8	491 ± 2.1	514 ± 12.3	523 ± 8.8	518 ± 3.5
60	516 ± 27.8	491 ± 8.5	527 ± 17.4	512 ± 17.4	527 ± 3.5
80	499 ± 2.1	510 ± 8.5	498 ± 2.1	580 ± 4.1	557 ± 12.3
Crop 2					
Total N (mg kg ⁻¹) (*ns)					
0	558 ± 8.5	618 ± 8.5	608 ± 12.5	583 ± 8.5	608 ± 40.9
20	565 ± 10.4	536 ± 7.5	550 ± 4.1	535 ± 6.5	573 ± 8.5
40	575 ± 21.8	533 ± 4.8	570 ± 14.7	583 ± 9.5	565 ± 18.5
60	608 ± 16.5	533 ± 8.7	573 ± 31.2	590 ± 4.1	533 ± 18.5
80	563 ± 8.5	535 ± 8.7	565 ± 14.4	525 ± 18.5	578 ± 4.8
Hydrolyzed N (mg kg ⁻¹) (*ns)					
0	474 ± 7.3	525 ± 7.3	516 ± 10.6	495 ± 7.3	516 ± 34.8
20	480 ± 8.8	444 ± 6.4	468 ± 3.5	455 ± 5.5	487 ± 7.3
40	489 ± 18.5	453 ± 4.1	485 ± 12.5	495 ± 8.0	480 ± 15.7
60	516 ± 14.0	453 ± 7.4	487 ± 26.5	502 ± 3.5	463 ± 15.7
80	478 ± 7.3	455 ± 7.4	480 ± 12.3	446 ± 15.7	491 ± 4.1
Crop 3					
Total N (mg kg ⁻¹) (*ns)					
0	550 ± 15.8	558 ± 48.7	545 ± 31.0	568 ± 22.5	573 ± 18.4
20	547 ± 21.7	545 ± 18.5	605 ± 15.5	543 ± 30.4	660 ± 55.8
40	585 ± 29.0	585 ± 34.8	595 ± 37.5	585 ± 30.1	565 ± 24.0
60	578 ± 18.0	585 ± 10.8	588 ± 14.4	578 ± 25.3	595 ± 24.0
80	565 ± 20.6	540 ± 10.8	608 ± 30.9	598 ± 12.5	578 ± 23.2
Hydrolyzed N (mg kg ⁻¹) (*ns)					
0	468 ± 13.4	474 ± 41.4	463 ± 26.3	482 ± 19.1	487 ± 15.7
20	457 ± 18.5	463 ± 15.7	514 ± 13.2	461 ± 25.8	548 ± 47.5
40	497 ± 24.7	497 ± 39.5	506 ± 31.9	497 ± 25.6	480 ± 20.4
60	491 ± 15.3	497 ± 9.2	499 ± 12.2	491 ± 21.5	506 ± 20.3
80	480 ± 17.5	451 ± 9.2	516 ± 26.3	508 ± 10.6	491 ± 19.7

⁽¹⁾ Quantity of hydrolyzed nitrogen in hydrolyzed neutral by the extraction in KCl 6.0 mol L⁻¹. (*ns) Not significant from Tukey test ($p \leq 0.05$).

fertilization in the three crops carried out, whose values are represented by the average of the three crops (Table 6). The values of significance when compared among the crops for these forms of organic N were 0.25, 0.32, 0.18, 0.11, and 0.16 mg kg⁻¹ for the forms N-NH₄⁺, hexosamine-N, amide-N, α-amino N and unidentified-N, respectively.

Among the assessed forms of organic N, the unidentified-N presented the highest contents in the soil (Table 6). This fraction is considered the to be the one with the highest recalcitrance in relation to other fractions evaluated, in which N is inserted into carbon chains with higher molecular weight and stability, and lower bioavailability in the soil (Camargo, Gianello, and Vidor 1997).

In the soil, organic N forms follow the following order of magnitude: unidentified-N > N-NH₄⁺ > amide-N > α-amino N > hexosamine-N (Table 6). It is highlighted that the unidentified forms of N and α-amino N represent the N forms with major stability and recalcitrance, while the forms of N-NH₄⁺, amide-N and hexosamine-N represent the organic forms N with less recalcitrance and higher bioavailability in the soil.

Probably, the increased presence of organic N in a more recalcitrant form is related to the lower speed of mineralization by soil microorganisms, in which these forms of N cannot be indicators of N availability to the plants in a short term. It can also add to these forms of organic N the part of hydrolyzed-N by the method (Table 5), characterized by the high level of complexity and occlusion in soil microaggregates.

In N forms with higher lability, N-NH₄⁺ has a higher proportion, which can be considered as the potential source of N available in the soil (Table 6). It is highlighted that hexosamine-N forms represent lower levels than other N sources, indicating higher bioavailability in the soil of this form of N. It can be inferred that, among the labile forms of organic N of hexosamine-N and amide-N,

Table 6. Organic nitrogen forms (mg kg⁻¹) of the soil after three sequential crops of millet in response to application of organomineral fertilizer in different doses of interaction between phosphorus and nitrogen (P × N).

Dose of P (kg ha ⁻¹)	Dose of N (kg ha ⁻¹)				
	0	50	100	150	200
N-NH₄⁺ (*ns)					
0	101 ± 5 ^{1/}	99 ± 2	105 ± 5	111 ± 3	110 ± 4
20	109 ± 3	112 ± 2	110 ± 1	104 ± 2	110 ± 2
40	109 ± 2	101 ± 5	117 ± 2	111 ± 1	115 ± 2
60	108 ± 2	102 ± 2	101 ± 4	113 ± 2	116 ± 2
80	105 ± 2	113 ± 2	106 ± 1	107 ± 2	114 ± 3
Hexosamine-N (*ns)					
0	15 ± 4	10 ± 2	15 ± 3	9 ± 2	13 ± 2
20	8 ± 1	11 ± 1	10 ± 1	9 ± 1	10 ± 2
40	10 ± 3	10 ± 2	10 ± 2	11 ± 1	12 ± 2
60	11 ± 2	10 ± 1	15 ± 3	10 ± 2	13 ± 3
80	15 ± 2	8 ± 1	9 ± 1	10 ± 1	11 ± 1
Amide-N (*ns)					
0	60 ± 2	56 ± 2	62 ± 2	61 ± 4	62 ± 2
20	62 ± 4	64 ± 3	61 ± 2	62 ± 3	62 ± 2
40	62 ± 3	57 ± 2	62 ± 3	59 ± 2	62 ± 3
60	63 ± 2	57 ± 3	62 ± 4	60 ± 2	60 ± 3
80	62 ± 3	60 ± 3	61 ± 2	60 ± 4	58 ± 2
α-amino N (*ns)					
0	17 ± 2	19 ± 3	21 ± 2	21 ± 2	17 ± 2 B
20	17 ± 2	22 ± 2	19 ± 2	20 ± 2	19 ± 2 B
40	22 ± 2 b	20 ± 2 b	20 ± 1 b	19 ± 2 b	30 ± 3 Aa
60	20 ± 3 b	20 ± 2 b	20 ± 2 b	23 ± 3 b	29 ± 3 Aa
80	19 ± 2 b	20 ± 2 b	22 ± 2 b	23 ± 2 b	29 ± 2 Aa
Unidentified N (*ns)					
0	293 ± 8	305 ± 23	303 ± 22	293 ± 15	304 ± 21
20	290 ± 10	289 ± 13	298 ± 11	283 ± 17	306 ± 22
40	292 ± 19	293 ± 16	296 ± 18	310 ± 14	281 ± 11
60	306 ± 19	293 ± 11	313 ± 20	292 ± 14	291 ± 12
80	285 ± 12	279 ± 11	298 ± 14	309 ± 12	304 ± 14

^{1/}Averages of the three crops. (*ns) Not significant from Tukey test (p ≤ 0.05).

with lower contents in relation to other fractions, they show less limitation to accessibility of decomposing microorganisms. It can be inferred that the simultaneous application of these elements in fertilization does not promote significant changes in soil N dynamics.

Thus, N and P in the soil did not present significant changes in their forms by the effect of the interaction between P \times N in different concentrations in organomineral fertilizers. These results indicate that the simultaneous application of these elements into the soil via fertilizers not interfered in their respective dynamics, under the conditions of this experiment.

In the plant, there was a response to the interaction between P \times N in organomineral fertilizers in the production of dry biomass (DB) of millet shoot, with higher response in the first crop (Table 7). The highest increases in the cultivation 1 occurred from the P \times N interaction of 20P \times 100N kg ha⁻¹, focusing on 80P \times 150N and 80P \times 200N kg ha⁻¹ interactions with the major DB values. This same effect is verified by the sum of DB production of the three crops performed. The same concentration of 20 kg ha⁻¹ in the organomineral fertilizer was the one that initiated the interaction in the increase of contents of P available in the soil (Table 4).

In crops 2 and 3, with lower DB production, there was only significant effect on crop 2 in the control treatment without application of P and N (0 \times 0 kg ha⁻¹) with the lowest DB values (Table 7). From crop 2, nutritional limitation can be occurring to the plants, especially by N in relation to P, showing appropriate levels of available P at higher doses (Table 4). It is highlighted that all other nutrients, except for the elements under test, were applied in large quantities in the soil to avoid deficiency in the plants during the three cultivations.

The effect of the interaction between P \times N on DB production in the first crop indicates that there is synergic effect of the simultaneous addition of these two elements in the soil. However, it is important to distinguish the processes of interaction or limitation between them, because of the simultaneous and individual additions. Regardless of limitation, there is a response to the crop performance. Simultaneous addition can add or not on the individual responses of each element (Duncan et al. 2018b; Harpole et al. 2011).

Both nutrients are important in plant nutrition and their deficiencies in the soil result in a significant reduction in the crop yield. The greater relevance of P can be explained by the fact that such nutrient is related to the structural components, and it regulates many processes in the plant, regulating the absorption of N, according to the demand of the plant growth rate (Duncan et al. 2018a; Groot et al. 2003). Therefore, P would act in the regulation of the demanded quantity of N that will be absorbed into the plant establishing the nature of needs.

Even though P is less required in relation to N in plant development, its strong interaction with the mineral phase of the soil causes the deficiency condition to be reached more quickly (Bolan et al. 2013), and its great importance in many biochemical and metabolic processes in plants ends up limiting plant growth more easily (Agren, Wetterstedt, and Billberger 2012; Groot et al. 2003). This would explain the higher response to N in doses from 20 kg ha⁻¹ of P, whereas for P, the higher responses occur at higher N rates due to the needs of plants for better growth.

For accumulation of P in the shoot, there was significant response to the fertilizers in the three crops with the highest contents in the first crop (Figure 2). In crop 1, the highest increases occur in doses of interaction between P \times N from 20P \times 150N kg ha⁻¹, which is the highest accumulation shown in the highest dose of 20P \times 200N kg ha⁻¹.

In crops 2 and 3 with lower P accumulation values, increases in the shoot occurred at interaction doses of 20P \times 200N and 20P \times 50N kg ha⁻¹ in crops 2 and 3, respectively (Figure 2). It can be noted that the highest increases in the P accumulation in the plant occur from the dose interaction of N is higher than 150 kg ha⁻¹ of N to organomineral fertilizers.

Particularly, the dose of 50 kg ha⁻¹ of N with organomineral fertilizers promotes an antagonistic effect on the accumulation of P in all doses (20, 40, 60, and 80 kg ha⁻¹), with a similar response to doses of P, without interaction of N in the fertilizer (Figure 2). Therefore, it can be inferred that the accumulation of P, the synergic effect of the doses of P, occurs from the interaction with the dose of 100 kg ha⁻¹ of N.



Table 7. Shoot dry biomass of millet in three sequential crops with 40 days each in Typic hapludox in response to application of organomineral fertilizer produced from poultry litter in different doses of interaction between phosphorus and nitrogen ($P \times N$).

Phosphorus (kg ha ⁻¹)	Dose (kg ha ⁻¹) Nitrogen			
	0	50	100	150
Crop 1 (g/pot)				
0	11.0 ± 0.5 Ca	14.9 ± 0.4 Ba	16.5 ± 1.4 Ba	16.8 ± 1.5 Ca
20	46.0 ± 2.8 Bb	47.6 ± 3.3 Ab	67.6 ± 3.1 Aa	76.8 ± 4.9 Ba
40	49.6 ± 1.2 Bc	57.6 ± 3.9 Abc	70.6 ± 1.1 Aab	80.0 ± 3.0 Ba
60	62.6 ± 4.0 Ab	59.4 ± 3.2 Ab	75.7 ± 4.9 Aa	74.6 ± 6.5 Ba
80	58.9 ± 2.3 Acd	48.5 ± 2.3 Ad	64.0 ± 1.2 Ac	84.3 ± 2.5 Aa
Crop 2 (g/pot)				
0	6.3 ± 0.3 Bb	7.9 ± 0.3 Aa	9.3 ± 0.2 Aa	9.9 ± 0.6 Aa
20	8.8 ± 0.3 Aa	8.9 ± 0.5 Aa	8.1 ± 0.3 Aa	9.4 ± 0.6 Aa
40	8.3 ± 0.2 Aa	8.4 ± 0.3 Aa	9.6 ± 0.6 Aa	8.8 ± 0.2 Aa
60	9.1 ± 0.4 Aa	7.9 ± 0.3 Aa	8.7 ± 0.4 Aa	9.3 ± 0.6 Aa
80	9.1 ± 0.2 Aa	8.7 ± 0.6 Aa	8.7 ± 0.5 Aa	9.2 ± 0.6 Aa
Crop 3 (g/pot)^(ns)				
0	5.6 ± 0.4	5.8 ± 0.4	5.8 ± 0.4	5.9 ± 0.2
20	6.7 ± 0.4	7.3 ± 0.2	7.7 ± 0.4	7.5 ± 1.4
40	7.2 ± 0.2	8.0 ± 0.7	7.2 ± 0.3	7.9 ± 0.5
60	7.8 ± 0.5	6.9 ± 0.5	6.8 ± 0.4	7.9 ± 0.2
80	8.1 ± 0.2	7.2 ± 0.3	7.4 ± 0.6	7.2 ± 0.9
Sum of crops (g/pot)				
0	22.7 ± 1.1 Ba	28.3 ± 1.0 Ba	31.3 ± 1.9 Ca	32.4 ± 2.4 Ca
20	61.5 ± 3.4 Ab	63.8 ± 4.1 Ab	83.4 ± 3.9 Aa	93.7 ± 6.7 Ba
40	65.2 ± 1.6 Ab	74.0 ± 4.9 Aab	87.4 ± 2.1 Aa	96.8 ± 3.8 Ba
60	79.5 ± 5.0 Ab	74.2 ± 3.9 Ab	91.2 ± 5.5 Aa	91.8 ± 7.2 Ba
80	76.3 ± 2.7 Ab	64.4 ± 3.3 Ab	70.1 ± 2.4 Bb	110.8 ± 4.9 Aa
				98.5 ± 3.4 Aa

Averages connected by distinct letters (lowercase letters in horizontal and uppercase in vertical) differ from Tukey test ($p \leq 0.05$). ^(ns) Not significant from Tukey test ($p \leq 0.05$).

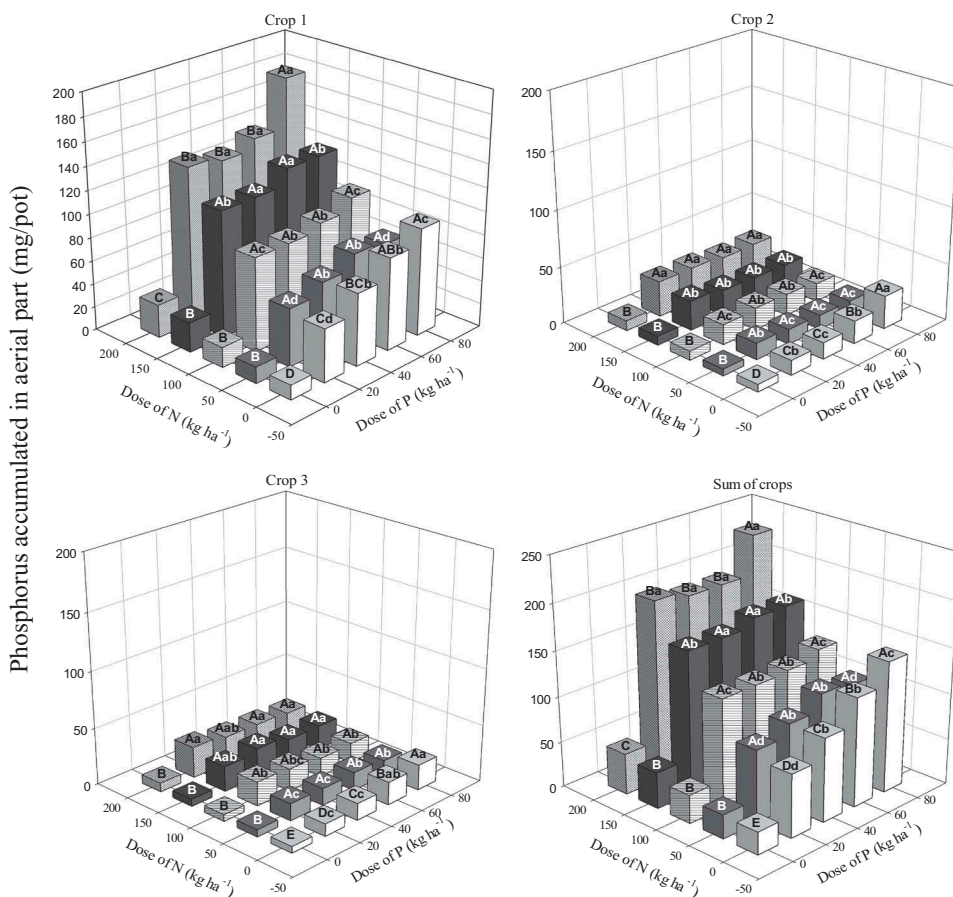


Figure 2. Phosphorus (P) accumulated in the shoot in three sequential millet crops with 40 days each in response to the application of organomineral fertilizer produced from poultry litter in different doses of interaction between phosphorus and nitrogen (P × N). Lowercase letters represent the difference between doses of N (axis “y”) in the dose of P and, uppercase letters represent the difference between the doses of P (axis “x”) in the dose N by Tukey test ($p \leq 0.05$).

In relation to the accumulation of P in the plants, the higher efficiency is showed by the doses of P, instead of N, applied through organomineral fertilizers, since it will determine the rate of demand for N, according to the plant growth rate directly or indirectly.

For the accumulation of N in the shoot, there was a significant effect of interaction in crops 1 and 3 (Figure 3). In crop 1 with higher values of N accumulation, there is an increase of N accumulation in the interaction doses of 40P × 50N kg ha⁻¹ with the highest accumulation shown in the highest dose of 80P × 200N kg ha⁻¹. This same effect is verified by the sum of the accumulations of the three crops. In crop 3, the interaction between P × N occurred at doses of 20P × 0N, showing the strong influence of P on the uptake of N when compared to the lack of N.

The higher absorption of N in the first crop can be justified, regardless of the concentration of interaction of the organomineral fertilizer, due to the forms of ammonium (N-NH₄⁺) and nitrate (N-NO₃⁻) are poorly absorbed by the charges of the soil colloid; and, when absorbed, they form less electrostatic energy bonds, prevailing in the net phase of the soil (Zhang et al. 2011), without restrictions for the absorption of the plant and presenting low residual effect.

For the accumulation of N in shoot, the highest efficiency is shown by N, instead of P. In the case of N, the results showed a strong influence of P in its absorption by the plants, even being an element absorbed in lower quantities, comparatively.

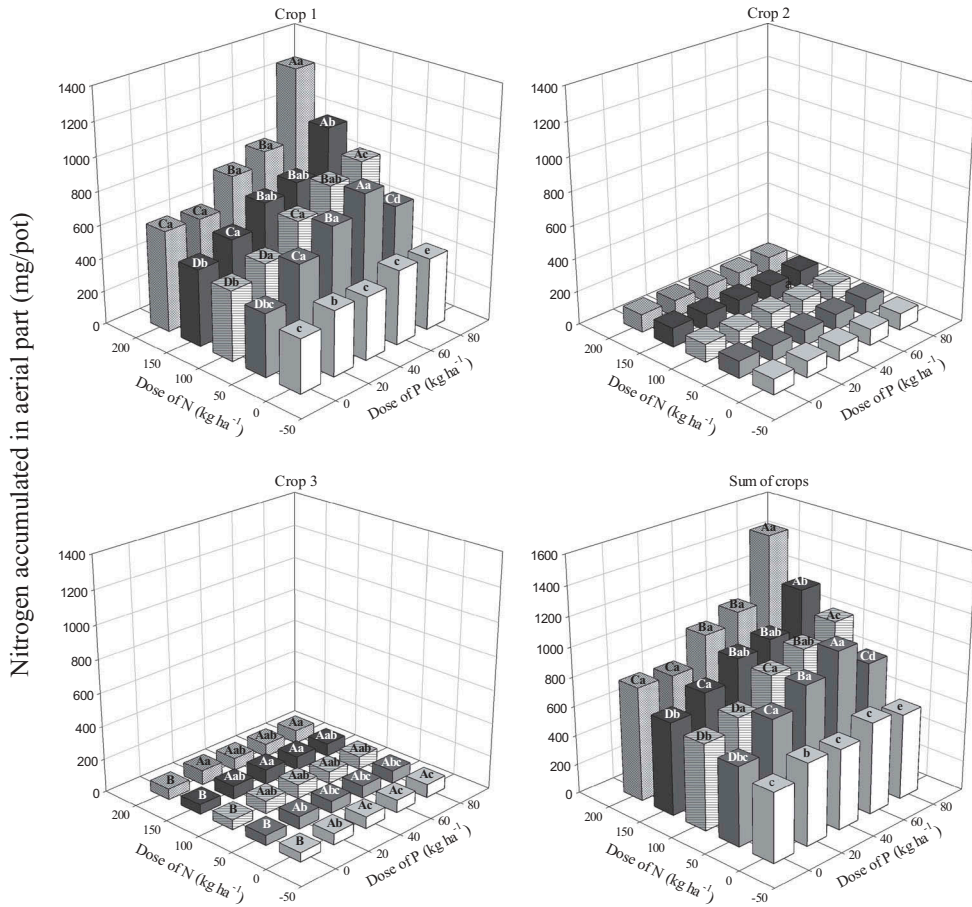


Figure 3. Nitrogen (N) accumulated in the aerial part of millet in three sequential crops with 40 days each in response to the application of organomineral fertilizer produced from poultry litter in different doses of interaction between phosphorus and nitrogen ($P \times N$). Lowercase letters represent the difference between doses of N (axis “y”) in the dose of P and, uppercase letters represent the difference between the doses of P (axis “x”) in the dose N by Tukey test ($p \leq 0.05$).

The importance of P for uptake and accumulation of N can be due to its importance in relation to many biochemical and metabolic processes in the plants. It is known that soil P limitation results in changes of N uptake by plants (Agren, Wetterstedt, and Billberger 2012; Duncan et al. 2018b). For such effect, there are many assumptions, which may be limitation of NO_3^- uptake by the roots as an active process that requires metabolic energy (Gniazdowska and Rychter 1999); redistribution and organ formation in lower concentration of N in the plant (Groot et al. 2003); or the decrease in the available metabolic energy in a lower concentration of ATP or lower activity of the ATPase enzyme (Gniazdowska and Rychter 2000; Rufty et al. 1993).

The effect of the $P \times N$ interaction by organomineral fertilizers for N and P is changed over the growing time in the plant, with distinct response patterns between the two elements. The interactions, provided between the elements and the concentration of each element in the interaction, promote, as a result, the synergistic or antagonistic combination, directly or indirectly influencing many soil and plant processes that interfere with the plant response.

In conditions of appropriate or high levels of P in the plant, there is an increased absorption and accumulation of N, while the increase of the dose of N promotes the absorption of P. Therefore, there is effect of $P \times N$ interaction by the simultaneous fertilization with a more evident effect on the

plants, instead of the dynamics of these elements in the soil. Therefore, the mechanisms involved in this interaction between these elements are not yet fully understood.

Conclusions

It can be inferred that simultaneous application of N and P does not promote significant changes in the dynamics of N in the soil, but the effect of the interaction changes over the cropping time in the plant, with different response patterns between the two elements.

In the soil the organomineral fertilizers do not show the effect of interaction between P × N on the forms of organic N, inorganic and organic P, with difference only in the available P.

In the plant, there is interaction between P × N in organomineral fertilizer in the shoot dry biomass of millet from the dose of 20P×100N, focusing on the dose of 50P×150N kg ha⁻¹, while the accumulation of P and N starts to occur from the dose of 40P×100N and 40P×50N kg ha⁻¹ with increase (synergy) in both nutrients up to the dose of 80P×200N kg ha⁻¹.

ORCID

Marco André Grohskopf  <http://orcid.org/0000-0002-6962-3359>

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