

Effect of phosphoric rock on the chemical, microbiological and enzymatic quality of poultry, equine and cattle manure compost mix

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Abstract

Purpose Phosphorus (P) is one of the key elements in the agricultural sector, allowing improved production yields. Phosphate rock is a natural source of phosphorus; however, its low reactivity limits the release of P available to plants in the short term, conditioning its application in a direct way. Research was conducted to determine the effect of phosphate rock on a manure compost mix by measuring its availability of P and its microorganism activity.

Method Cattle manure, equine manure, and poultry manure from three provinces of the department of Boyacá, Colombia, were moistened up to 60% with fermented mineralized liquid and composted with different proportions of phosphate rock. After 60 days of composting, the mineral content and microorganism activity were measured.

Results This study revealed three notable results. First, the addition of phosphate rock led to an increase in total and available P and a decrease in water-soluble phosphorus and inhibitory effects of phosphatase activity. Second, composting with the three manures resulted in microorganism activity levels higher than 700×10^4 CFU, exceeding the NTC's definition of an inoculant fertilizer in the agricultural process. Third, a strong positive relationship was found between ammonia-oxidizing bacteria and PBII, a medium correlation was found between PSM and TP, and a negative correlation was found between pH and PT.

Conclusion Composting manure with phosphoric rock could be a low-cost source of macronutrients, minerals, and microorganisms to promote soil health and crop yields.

Keywords Composting, Phosphate rock, Manure, Phosphate solubilizing

Introduction

Phosphorus (P) is an essential element of energy and transport processes for the growth and development of plants and the correct functioning of soil microorganisms (Ramaekers et al. 2010; Ditta and Khalid 2016). The majority of P is applied to agroecosystems in the form of phosphatidic mineral fertilizers, but it is re-

tained in the soil by the processes of fixation / precipitation with clays by interactions with hydroxyl groups (OH⁻) at acid pH. The presence of Ca²⁺ y Mg²⁺ gives a second possibility of fixation through the cations absorbed by the clay at basic pH, which act as bridges with the phosphate anions (Lemanowicz 2018). Further, the high capacity of the P ions generating complex mono, di and multi-dentate bonds with various metal cations such as Fe and Al can convert P into forms not available to plants (Hinsinger 2001; Condrón et al. 2013; Schütze et al. 2020).

Phosphate rock (PR) is a low-cost natural source of phosphate. However, phosphate contained in the structure of the PR is not water-soluble and cannot be absorbed directly by plants (Khan et al. 2012; Yu et al. 2012; Ditta et al. 2018). To achieve a form of phosphorus that can be absorbed directly by plants, solubilization with inorganic acids such as H₂SO₄, HNO₃ o H₃PO₄ is required. However, this strategy can result in phosphorus that is not absorbed by fixation or leach-

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ing. Consequently, different strategies, such as the use of phosphate solubilizing microorganisms, growth-promoting rhizobacteria, and composting have been proposed to increase the availability of P existing in PR.

Composting allows the decomposition of organic matter through biological degradation under controlled conditions of temperature, moisture, and C/N ratio. These three factors regulate the growth of aerobic microorganisms responsible for creating material rich in (i) stable organic matter, (ii) minerals and competitive microbial biomass as microorganisms, and (iii) phosphate solubilizers and nitrogen fixers, all of which may be used as a potential resource in agricultural and horticultural soils (Huang et al. 2000; Gerba and Pepper 2019; Wei et al. 2021).

Composting enriched with phosphate rock (C-PR) has been proposed as a promising procedure because during the degradation of organic material (manure, plant material residues from agro-industrial processes, or food residues), the microorganisms solubilize the added P from organic materials such as phospholipids, amino phosphates and phytins and from the phosphate rock. Different studies have shown that the availability of P from PR can be increased during the composting process (Singh and Amberger 1991; Chandra et al. 2020). For example, Biswas and Narayanasamy (2006) found that the composting process increased the availability of P from PR absorbed by beans. Recently, in the study conducted by Ditta et al. (2017) composted fruit peel residues and pea residues enriched with different proportions of phosphate rock and analyzed the phosphate rock's effect on the growth and yield of chickpea inoculated with phosphate solubilizing microorganisms. Results indicated that a rise in the proportion of PR increases the availability of P, presenting the maximum value with a proportion of 50:50. These studies show that it is possible to increase the yield of grain and chickpea biomass by adding organic fertilizers enriched with cheap P sources.

As mentioned above, C-PR is a cost-effective alternative to progressively return organic materials and essential nutrients to the soil using low-cost raw materials (agricultural and food residues). Additionally, C-PR should be considered an economical P fertilizer in harvest production. Nevertheless, to understand C-PR's impact on soil components such as P, minerals, and microorganisms, it is necessary to know their distribution. Therefore, the objective of this study was to determine the effect of additional PR on the content

of total P, available P, P-solubilizing microorganisms, phosphatases, and ammonia and nitrate-oxidizing bacteria in the compost mixture comprised of cattle, equine and poultry manure enriched with three proportions of PR and minerals in the form of sulphates, in the Supatá village located in the Ventaquemada in Boyacá, Colombia municipality.

Materials and methods

Area of study

Research was carried out in the Supatá village of Ventaquemada in the state of Boyacá, Colombia, at the facilities of the company Victoria Granja Agroecológica SAS, a company dedicated to the agricultural exploration and commercial production of organic fertilizers. Ventaquemada has an approximate temperature of 281.15 to 287.15 K, an altitude of 2,630 meters above sea level, average annual rainfall of 1,367 millimeters, and a relative humidity of 70 to 90%.

Preparation of phosphate rock-enriched compost

Cattle manure was collected in a dairy farm located in Ventaquemada in the state of Boyacá, Colombia. Equine manure was obtained from an equine farm located in Zipaquirá in the state of Cundinamarca, Colombia. Poultry manure was collected from laying hen sheds located in Soracá in the state of Boyacá, Colombia. The main chemical properties of these materials are shown in section A.2, Table 1. Manures without any type of treatment (with a particle size of approximately 1 cm) were taken to a 10x15 m² composting area, placed into piles measuring approximately 3 m in diameter and 1.5 m in height at the center of the pile (Fig. 1), and covered with plastic to maintain the temperature of the composting process and avoid direct rainfall.

The composting process was carried out as follows: approximately 14.2 tons of cattle, equine and poultry manure were mixed in a 1:1:0.26 ratio and moistened to 60% with liquid fermentation prepared from fresh cattle manure previously mineralized with K, Mg, Cu, Fe, Mn, Zn and H₃BO₄ sulfates in a concentration of 0.5% each (Fig. 2a). The liquid fermentation was added to enrich the compost with micronutrients that contribute to the growth and reproduction of the plant. For example, Mn contributes to the synthesis of hormones, proteins, fats, and the assimilation of N; Zn helps in the secre-

tion of enzymes and biosynthesis of proteins responsible for regulating plant growth; Cu and Fe are part of the metabolism process of plants (Salisbury and Ross 1992; Wang et al. 2004); and Ca and Fe play a role in the formation of soil aggregates, generation of acidity or alkalinity, and feeding of microorganisms. Then, the compost material was divided into 12 piles weighing 1/4 ton each (Fig. 2b). The procedure for the preparation of mineralized fermentation and its chemical properties is mentioned in the supplementary material (A.1). The distributed material (Fig. 2b) was then enriched with different amounts (5%, 10% and 15%) of commercial phosphate rock (22% of P_2O_5). With the piles

carefully mixed into four equal piles, the material was composted for 35 days at a controlled temperature (5% PR mix averaged 303.15, 10% PR mix averaged 333.15 K, and 15% PR mix averaged 293.15 K) and moisture (initially at 60% and ending at 20% w/v), and were turned over weekly by hand. Once the process was finished, samples were taken for analysis. Composting of the control group (mixture of cattle manure, equine and poultry manure without PR) was not carried out in this manner since the percentages of total phosphorus in the control group ranged from 0,5-1,0 de P_2O_5 %, well below that required by the Colombian Technical Standard (CTS) for mineral organic fertilizer.



Fig. 1 Composting area in the Victoria Granja Agroecológica SAS company



Fig. 2 (a) Mix of cattle manure, equine manure, and poultry manure (1:1:0.26) moistened with mineral fermentation. (b) Distribution of the manure mixture

Chemical and microbiological properties

After 35 days of composting, sub-samples from each pile were collected at depths of 20, 50 and 100 cm (10

total sub-samples equaling approximately 500 g of C-PR), mixed, and packed in hermetic seal plastic bags for analysis. Chemical analysis was carried out in the laboratories of the Research and Innovation in the Materials Science

and Technology Institute (RIMSTI) and the teaching laboratory of the Faculty of Agrarian Sciences of the Pedagogical and Technological University of Colombia, Tunja headquarters (UPTC). Quantification of solubilizing phosphate and nitrogen-fixing microorganisms was carried out in the Environmental Biology laboratory of the same university. Phosphatase's activity was analyzed by the Interdisciplinary Group of Molecular Studies (IGMS) from Universidad de Antioquia in Medellín, Colombia. Each analysis was performed in duplicate.

The total and assimilable P were determined by following the guidelines of the CTS 234 Standard. Water-soluble P was determined by following the method reported by Hanson (1950) and the available P was performed using the Bray II method (Bray and Kurtz 1945). The content of K, Ca, Na and Mg was carried out in a spectrophotometer of atomic absorption (Thermo Scientific Model 3000). The microbiological analysis to determine the populations of phosphate solubilizing microorganisms, ammonia-oxidizing bacteria, and nitrate-oxidizing bacteria was carried out using the technique of serial dilutions and sowing in solid culture media PVK® and TSA® (Pikovskaya 1948).

For the determination of the enzymatic activity of phosphatase, the methodology reported by Tabatabai (1994) was followed and the population density was performed by the direct method of quantification with artificial substrates of fast hydrolysis, such as p-nitrophenyl phosphate (PNPP), which is hydrolyzed until p-nitrophenol (PNP) develops a yellow color in a basic medium that is susceptible to colorimetric determination at 405 nm done in the Interdisciplinary Group of Molecular Studies GIEM.

Statistical analysis

The data were tabulated in Excel® version 2019 by performing tests of homogeneity of variance with the Bartlett method and normality with the Shapiro-Wilk method. The ANOVA test was performed determining the statistical differences between samples and the Tukey mean comparison test was performed with a significance level of $P < 0.05$, by means of the R program version 4.0.1 using the agricultural package "Library agricolae". Finally, each of the variables was treated with the Performance Analytics statistical package, a variance correlation performed through the R program version 4.0.0 to observe the correlation of the variables studied in relation to the different forms of phosphorus.

Results and discussion

Effect of temperature and pH

The temperature observed in each of the piles presented similar values and normal behavior for composting (Fig. 3). The average temperature of the three PR mixtures started at 288.15 K and increased to approximately 334.15 K in the first week. Subsequently, the average temperature of the three PR mixtures decreased to 322.15 K in the second week, 308.15 K in the third week, and 303.15 K in the fourth week; complying with the parameters established in NTC 5167 (2001). Temperature data indicate that the addition of PR does not significantly affect the metabolic activity of the microorganisms in each composting phase. This result is similar to the findings reported by Lu et al. (2014), who composted pig manure and rice straw enriched with different proportions of PR (2.5, 5 and 7.5%). However, authors such as Zhan et al. (2021) and Oviedo-Ocaña et al. (2021) found that the addition of the PR does affect temperature by increasing the air-filled porosity and providing O₂ that promotes aerobic fermentation (results obtained using raw material kitchen waste and sawdust ratio of 4:1 and a mix of green waste: unprocessed food waste: processed food waste: sawdust: PR in proportions 46:19:18:13:4, respectively). Finally, temperature reached in the thermophilic phase falls in the range of temperature for the elimination of pathogenic organisms, guaranteeing the safety of the final product.

The pH values measured in the final stage of composting are close to neutral. There was a slight pH decrease associated with the increase in PR concentration (A.2, Table 2), presenting the following order: 5% PR > 10% PR > 15% PR. The increase in PR could favor the production of acidic species such as organic acids and phenolic compounds, which would be contributing to the decrease in pH (Satisha and Devarajan 2007). Our results indicate a different behavior from that reported by Bustamante et al. (2016) and Oviedo-Ocaña et al. (2021). Satisha and Devarajan's study observed a pH of 7.42 in absence of PR and a pH of 7.50 with 4.6% of PR, results obtained by composting vegetable waste from palm pruning (*Phoenix sp.* and *Washingtonia sp.*), grass (*Lolium perenne L*), and a 1:1 mixture of olive tree pruning (*Olea europaea L*) and conifer (*Pinus sp* and *Picea sp*) in a 32:35:33 ratio, respectively. Bustamante and Oviedo-Ocaña's study indicates a direct rela-

tion between the final pH with PR concentration (higher PR% equals higher final pH), with material yielding pHs greater than 8 when enriched with 5%, 10% and 15% PR. The difference in the results of our study versus Satisha and Devarajan's study and Bustamante and Ovideo- Ocaña's study may indicate that the composition of the starting material influences the production of acidifying substances such as organic acids, and consequently, the possible interactions that may occur in the solubilization of the P present in the PR through

its hydroxyl and carboxyl groups, which generates changes in pH. In addition, there is a possibility that the variability of the pH could be associated with the volatilization of products, such as CO_2 , which would prevent the formation of carbonic acid (Moharana et al. 2018). Additionally, the pH values obtained for each of our study's PR samples are within the range established in NTC 5167 (2001) to be considered a high quality and stabilized organic material.

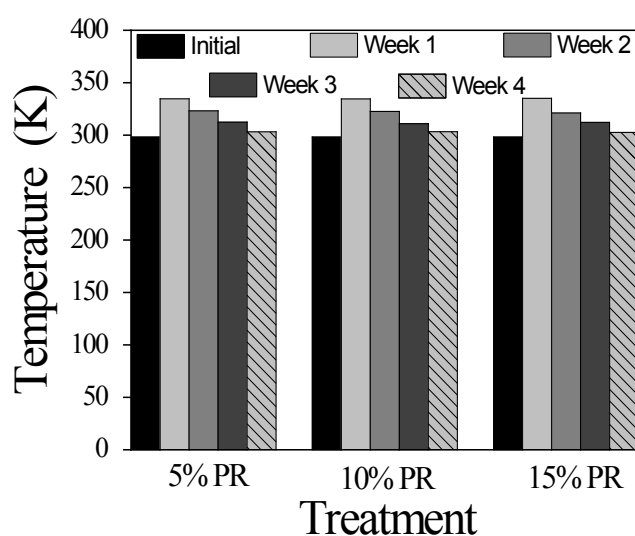


Fig. 3 Variation in compost temperature with different concentrations of PR

Effect on the behavior of phosphorus

The amount of total P (TP), available P (P_2O_5), P Bray II (PBII), and water-soluble P (Psw) are displayed in Fig. 4. Previous studies found a progressive increase in TP with increasing PR. Bangar et al. (1985) found a 32% increase in TP resulting from 10% and 15% of Mussoorie PR when composting a mixture of agricultural residues, cattle manure, soil, and composted material for 30 days. Lu et al. (2014) indicates a 16.9% increase in TP when PR is increased from 5% to 7.5%. Ditta et al. (2018) increased TP by 14.9% when increasing PR from 25% to 50% (22% P_2O_5) on a compost mix consisting of fruit peels and vegetable waste. Montoya et al. (2019) increased TP by 48.6% when composting a mixture of coffee peel, banana peel, cattle manure, and food waste with 15% of PR versus a control sample. In addition, Zhan et al. (2021) observed that when composting kitchen waste with sawdust (in a 4:1 ratio) for 30 days with different PR ratios (5%, 10%, and 15%), TP increased by 50% for all samples. In our study, an increase of 8.36% and 33.3% was observed when in-

creasing PR from 5% to 10% and from 10% to 15%, respectively, a behavior similar to that reported by Bangar et al. (1985). Additionally, the behavior of P Bray II and the percentage of available P determined by CTS 234 was directly proportional to the concentration of PR in the following order: 10% > 15% > 5% (Fig. 4), which differs from the findings of Kutu et al. (2018), in which available P decreased with proportions greater than 20% of PR in the maturation stage. The difference in the results of our study compared to those mentioned in this paragraph can be attributed to the composition of raw materials used, composting times, chemical quality of the raw materials used, and compost methodology.

Samples enriched with PR resulted in the following order of Psw content: 10% > 15% > 5% (Fig. 4). These results agree with that reported by Kutu et al. (2018), who observed a decrease of approximately 17% of Psw when a ratio of 7:3 to 5:5 manures: PR was passed in the maturation phase. The decrease in Psw concentration with the increase in PR may be explained by possible reactions of P and PR components (Singh 1985) with dolomite lime and minerals added to composting.

Authors such as Singh described that small amount (1 g) of Mussoorie PR can immobilize up to 22 mg of soluble P. Furthermore, it is possible that this behavior is due to the mass dilution effect mentioned by Bangar et al. (1985). In our study, we observed that the decrease in Psw occurred with concentrations higher than 10% of PR (0.6075 mg / kg with 10% PR and 0.5450 mg / kg with 15% PR, a decrease of 10.3%). On the other hand, increasing PR from 5% to 10% resulted in an increase of 40.3% of Psw. Similar results were described by

Kutu et al. (2018), where an increase in PR produced slight changes in Psw. However, when tests were carried out using the highest proportion of manure to PR (5:5), Psw concentration decreased by approximately 19% compared to a lower manure to PR ratio of 7:3. In addition, no statistically significant differences were observed for TP, available P and P-BII with the addition of PR. However, Psw presented significant differences with 5% PR compared with 10% and 15%, respectively.

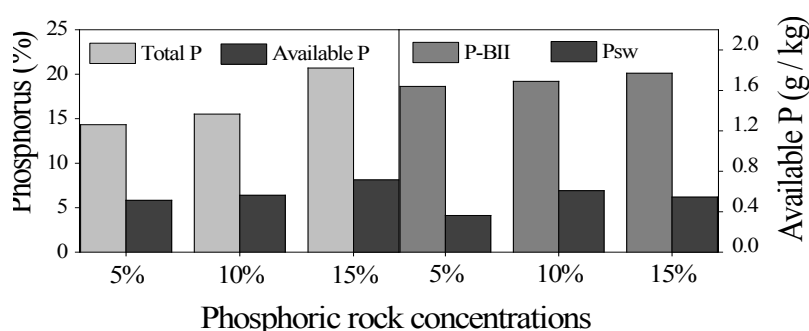


Fig. 4 Variation of total P, assimilable P, and water-soluble P at different PR concentrations

Data fluctuation of each test (A.2, Table 2) may be related to a heterogeneous behavior in microbial activity, which could be assumed as a normal behavior because the process of solubilization of P is considered a complex phenomenon, which can be considerably affected by temperature, pH, oxygen concentration, and humidity, among others (Chen et al. 2006; Zhu et al. 2012). In this sense, a relationship of different bacterial communities could influence the solubilization of P present in organic matter and in PR by the different mechanisms proposed such as the generation of organic acids (Wei et al. 2018).

Theories for solubilizing inorganic P

Different theories have been proposed to explain the solubilization of inorganic P. Some of the most well-recognized theories are the production of organic acids (Seshachala and Tallapragada 2012; Billah et al. 2020), inorganic acids (Sharma et al. 2013; Shrivastava et al. 2018), acidification by H^+ (Goldstein 1994), production of chelating substances or siderophores (Rodríguez and Fraga 1999; Toscano-Verduzco et al. 2020), assimilation of NH_4^+ within microbial cells (Sharma et al. 2013), enzymes release or enzymolysis (Zhu et al. 2012) and by the presence of CO_2 (Rodríguez and

Fraga 1999; Moharana et al. 2018). We believe that the decomposition of PR to convert unavailable P into available P is mainly influenced by the generation of short chain organic acids (AOCC) such as acetate, lactate, oxalate, tartrate, succinate, citrate, gluconate, ketogluconate and glycolate (Hellal et al. 2012; Ditta et al. 2018), which shifts the reaction to the right, complying with the Le Chatelier principle that can be produced in the different stages of composting by the presence of microorganisms such as *Bacillus*, *Arthrobacter*, *Penicillium*, *Aspergillus*, *Micrococcus* and *Streptomyces*, which has been shown to be existing in equine and cattle manure composting processes (Boulter et al. 2002; MacCready et al. 2013). On the other hand, the formation of carbonic acid produced by the presence of CO_2 (produced during composting) and water at a low pH also contributes to increase available P from PR (Chien 1979; Biswas and Narayanasamy 2006; Lu et al. 2014). As a result, future studies are necessary to investigate and observe the behavior of acids which can improve the availability of P from PR.

Effect on the behavior of Na

In the case of sodium, its existence in raw materials was associated with the intake of mineral salts by different

animals (cattle, horses, and poultry). The highest Na value is presented by poultry manure and can be linked to the cleaning and disinfection processes of poultry farms that typically use disinfectants such as NaClO, which can increase the amount of Na in the manure (Onwosi et al. 2017; Liu et al. 2018). The Na content did not show significant differences in our study – only a slight decrease between 1.5 and 3% was found. We believe that this could be related to the processes of mineral assimilation by microorganisms and their need to take micronutrients such as Na as part of their energy metabolism to carry out and favor biochemical processes in the degradation of organic matter (Liu et al. 2018). In conclusion, an increase in the amount of PR did not significantly affect the Na concentration in the final composted product. The behavior of Na can be observed in Fig. 5.

Effect on the behavior of Ca, K and Mg

Higher concentrations of Ca and Mg were observed in our study with proportions that ranged from approximately 20% to 25%, compared with the raw materials that presented values below 2.2% mg / kg. The significant increase in these two minerals can be attributed to the addition of dolomite lime (59% CaCO₃ and 33% MgCO₃) and PR (36% CaO). A similar behavior was observed for K, resulting in values between 10% and 14% in the final compost samples, compared to initial values ranging from 0.84% to 2.07%. Variation in the concentrations of these elements and the increase in the final product can be attributed to the heterogeneous losses of mass during the decomposition of organic matter. The data on the Ca, Mg and K content do not show significant statistical differences; therefore, like the Na content, an increase in the concentration of PR did not affect the availability of these nutrients. The behavior of Ca, K and Mg can be observed in Fig.

5. In conclusion, no statistically significant differences were observed for Na, K, Ca and Mg between the samples.

Bacterial behavior (BBac)

The behavior of microorganisms such as fungi, actinomycetes, and especially bacteria are affected in the different phases of composting with the addition of phosphate rock. This indicates that the variability in the number of bacteria (CFU g⁻¹) could affect nutrient availability. Kutu et al. (2018), observed that in the final stage of composting (maturation phase), the bacterial community decreases when handling PR concentrations between 10% and 20%; however, an inverse effect was observed at concentrations greater than 20%, showing a gradual increase in CFU g⁻¹. In our study, a 10.4% increase in the bacterial community was observed when increasing PR from 5% to 10%, but increasing PR from 10% to 15% resulted in a decrease of 4.3% in the bacterial community. The results could be associated with the conditions in which the composting was carried out, such as temperature, moisture, aeration, turning over, and components of the starting material, and mineral additives, which can exert different effects on the diversity and distribution of bacteria during the composting process. Additionally, investigations show that the maturation stage is the point of the greatest presence of bacteria; this behavior is associated with re-colonization processes of thermotolerant microorganisms from the thermophilic phase (Scheuerell and Mahaffee 2013; Partanen et al. 2010; Villar et al. 2016). Finally, samples yielded values higher than 700*10⁴ CFU, within the established range (between 100 to 10,000 million CFU) to consider compost as an inoculant material in agricultural procedures (NTC 1927 2001): fertilizers and soil conditioners). In conclusion, no statistically significant differences were observed in the Bbac's CFU.

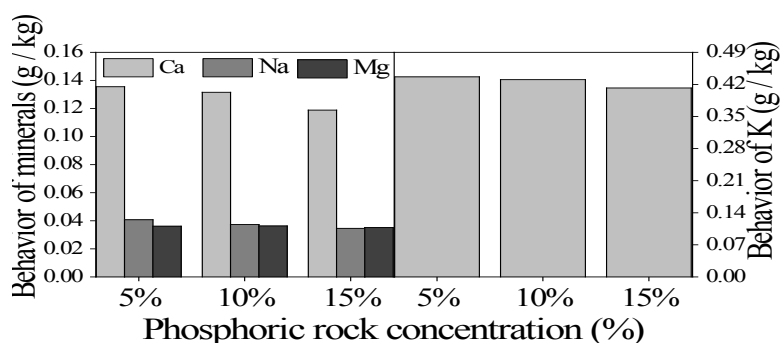


Fig. 5 Variation of the behavior of minerals at different PR concentrations

Effect on phosphate solubilizing microorganisms (PSM)

Microbial diversity in composting plays an important role in the availability of nutrients present in organic and inorganic sources. Different studies have established that the population and diversity of PSM are strongly influenced by environments rich in organic matter, favoring microbial growth and the solubilization/mineralization of organic and inorganic P, leaving it chemically available to be absorbed by plants (Khan et al. 2009; Zhang et al. 2014). Microbial diversity can also be affected by pH, temperature, moisture, metals, and the addition of sources, such as PR, to enrich the composted material. Our study shows a 21.8% increase in CFU/g when increasing PR from 5% to 10% and a 10.7% increase in CFU/g when increasing PR from 10% to 15%. These findings are in accordance with that reported by Priyal et al. (2011), who observed an increase in PSM density in soil with the addition of compost enriched with PR, possibly related to a greater availability of C, N and P to be used by the microbial community. Additionally, the 10.7% increase in CFU/g going from 10 to 15% of PR could be explained by a slight decrease in the degradation of organic compounds due to the growth inhibition processes in the bacterial growth of PSM by the addition of high concentrations of PR, as observed in a previous study (Wei et al. 2018), where addition of tricalcium phosphate resulted in a decrease of 46% by adding 2.5 w/w. Finally, a low correlation was observed between PSM and Psw with the highest concentration of PR (Table 2), which can be explained by the different reactions of organic and inorganic phosphorus with minerals as Ca, Fe, Mg and others; resulting in species poorly soluble in an aqueous medium with inhibitory effects on enzyme activity (Wei et al. 2018).

It is worth noting that the mineralized organic fertilizer produced by this study provides organic matter with macronutrients and minerals that can serve as food for soil microorganisms and improve microbial activity, thus promoting a better development of the crop due to the greater availability of nutrients such as NPK. Furthermore, phosphatases activity reported in this study indicates mineralization of the organic P present in the compost and reported as part of total P may be continuing in the soil with the available and unavailable inorganic P from the PR. In conclusion, no statistically significant differences were observed in PSM population.

Effect on phosphatase activity (PA)

Our results showed an inhibitory effect on the activity of phosphatases with the increase in PR concentration (Table 1). This result agrees with that proposed by Kutu et al. (2018), who observed a reduction of approximately 16% when increasing PR concentration from 10% to 20%, remarkably close to the 15% found in our study when PR concentration increased from 10% to 15%. This finding could be associated with a lower content of organic matter in samples with a higher amount of PR, implying a lower abundance of potentially degradable substances that favor extracellular enzymatic activities through the formation of more available enzyme complexes, which would agree with the study of Chang et al. (2007), who found that enzymatic activity has a linear behavior with the content of organic matter. Additionally, it is possible that the increase in the concentration of soluble P (Fig. 4) could affect the activity of acid phosphatase, according to previous studies that found inhibitory effects among the production of phosphatase and soluble P from fluorapatite and rock phosphate from Lalitpur (Shrivastava et al. 2011; Nahas and Assis 1992), which could be associated with competitive inhibition processes due to the presence of PO_4^{3-} or the negative feedback of PO_4^{3-} ions in the PHO gene, generating a decrease in phosphatase synthesis by microorganisms (Oshima et al. 1996; Criquet et al. 2007). In conclusion, no statistically significant differences were observed in PA activity.

Effect on oxidizing ammonia and nitrate bacteria

The organic nitrogen present in proteins, amino acids, and nucleic acids is decomposed by the presence of bacterial communities, transformed to NH_3 by ammonia-oxidizing bacteria (AOB) (Bernal et al. 2009), and subsequently oxidized to nitrate by nitrate-oxidizing bacteria (NOB) in two stages (Maeda et al. 2010). The most probable number (MPN) technique was used to determine the population density of AOB and NOB, given their influence on the nitrogen cycle in the composting processes. Results are shown in Table 1. The population counting of AOB differed slightly with PR increases: a 5% increase in population density of AOB was observed when PR concentration was increased from 5% to 15%. Similar behavior was not observed in NOB, where the results of each sample were essentially equal. Research indicating that the addition of P affects

the distribution of bacteria responsible for the N cycle but does not significantly affect their population density (Tang et al. 2016; Lage et al. 2010) was confirmed in this study. Consequently, future studies should investigate the behavior of AOB and NOB in different stages of the pro-

cess using molecular techniques to observe their behavior and demonstrate the effect of PR on the distribution of bacterial communities. In conclusion, statistically significant differences were observed for ammonia-oxidizing bacteria but not for nitrite oxidizing bacteria.

Table 1 Microbiological activity and phosphatase of the composting of manure C: E: P mixture with different PR concentrations

Test	BBac (UFC/g)*10 ⁴	PSM (UFC/g) *10 ²	PA (mmol / g*min)	AOB (NPM/g)	NOB (NPM/g)
5%	621.25	53.75	0.1030	90.0	46.5
10%	730.00	65.50	0.0740	94.5	45.0
15%	545.00	72.50	0.0753	95.0	45.0

Bacterial behavior (BBac), Phosphate solubilizing microorganisms (PSM), Phosphatase activity (PA), ammonia oxidizing bacteria (AOB) and nitrate oxidizing bacteria (NOB).

Statistical analysis

Chemical properties were combined and analyzed for correlations regardless of compost type (Fig. 6). The

analysis of the correlation index from the value of r from the different parameters studied was carried out considering the following aspects: if $r < |0.1|$, the correlation is negligible; if $|0.1| < r \leq |0.3|$, the cor-

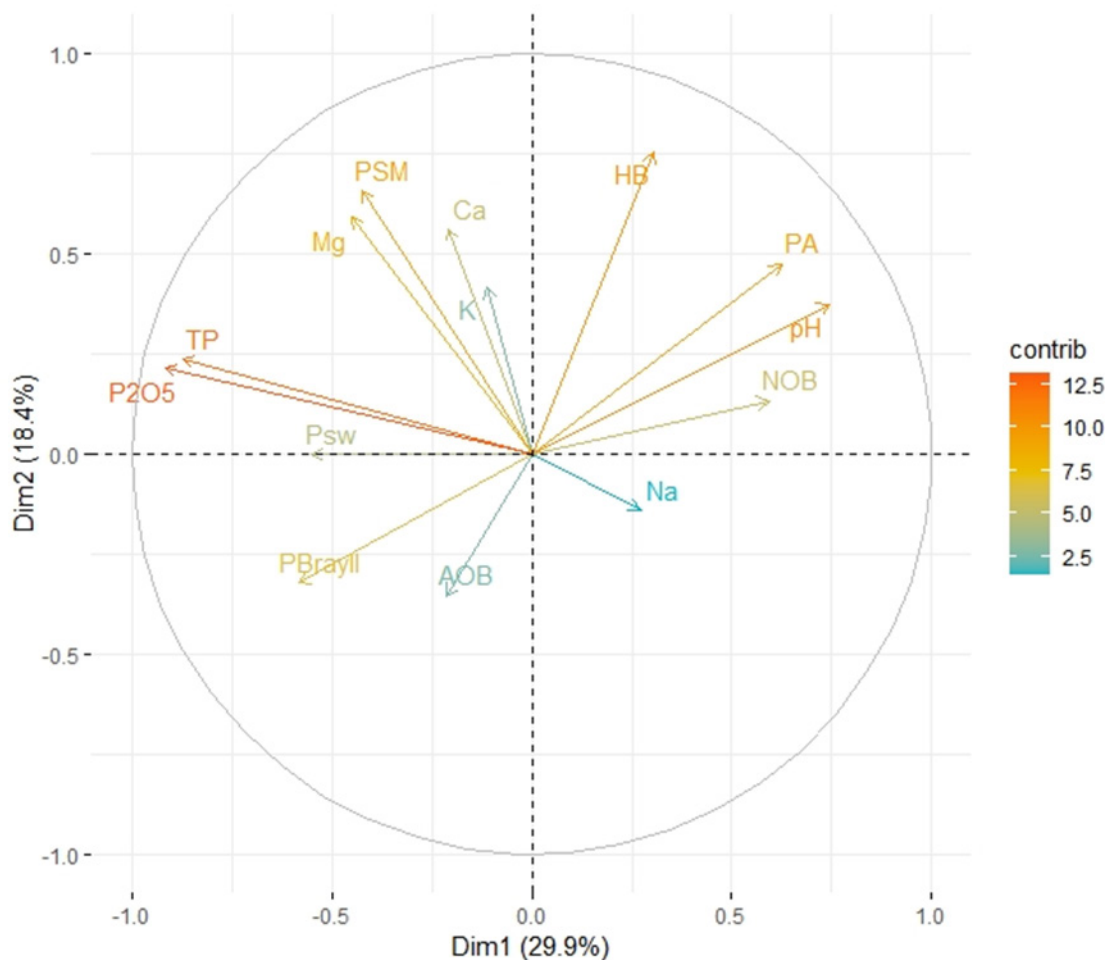


Fig. 6 Analysis of main components in manures composting with different PR concentrations

Table 2 Pearson's correlation of the main components in manures composting with different PR concentrations

	pH	TP	P ₂ O ₅	PBII	Psw	Ca	Na	K	Mg	BBac	PSM	PA	AOB	NOB
pH	1													
TP	-0.607	1												
P ₂ O ₅	-0.592	0.933	1											
PBII	-0.455	0.333	0.429	1										
Psw	-0.156	0.366	0.355	0.336	1									
Ca	0.116	0.331	0.417	0.074	-0.149	1								
Na	0.210	-0.225	-0.291	0.122	-0.139	0.330	1							
K	0.116	0.040	0.065	0.345	-0.032	0.263	0.109	1						
Mg	-0.257	0.446	0.445	0.104	0.065	0.498	-0.279	0.384	1					
BBac	0.449	-0.106	-0.235	-0.430	0.114	0.142	0.030	0.142	0.369	1				
PSM	0.013	0.546	0.570	-0.008	0.447	0.283	-0.165	0.230	0.239	0.411	1			
PA	0.707	-0.411	-0.418	-0.207	-0.368	0.148	0.034	0.266	-0.117	0.475	0.031	1		
AOB	-0.151	0.077	0.081	0.770	0.251	0.099	0.532	0.090	-0.056	-0.191	-0.130	-0.150	1	
NOB	0.338	-0.337	-0.432	-0.268	-0.557	0.089	0.355	-0.098	-0.128	0.301	-0.092	0.400	0.202	1

Total P (TP), P Bray II (PBII) and water-soluble P (Psw), Bacterial behavior (BBac), Phosphate solubilizing microorganisms (PSM), Phosphatase activity (PA), ammonia oxidizing bacteria (AOB) and nitrate oxidizing bacteria (NOB)

relation is low; if $|0.3| < r \leq |0.5|$, the correlation is medium; and if $r > |0.5|$, the correlation is high or strong. The r values are shown in Table 2. TP shows a strong positive correlation with P_2O_5 and a medium correlation with PSM. PSM with P_2O_5 show a medium correlation. PBII shows a strong correlation with AOB and a medium correlation with Na. pH shows a medium negative correlation with TP and P_2O_5 . NOB shows a negative correlation with Psw. The positive correlation between PSM, P_2O_5 and PBII can be attributed to an environment rich in organic matter favoring microbial growth and solubilization of organic and inorganic P (Bolívar-Anillo et al. 2016). The strong positive correlation between AOB with PBII could be related to the oxidation of NH_3 to NO_2 in the first stage (nitrification) where H^+ is released, contributing to the acidity of the environment, and favoring the solubilization of P from the PR. Also, this phosphorus can be accumulated by AOBs in their organisms, as mentioned Liu et al. (2018).

The strong negative correlation between pH and P (TP and P_2O_5) could be explained by the fact that an increase in PR favors the production of acidic species as explained in previous sections, which generates a decrease in pH by increasing the H^+ concentration in the environment. In addition, the strong positive correlation between PA and pH can be explained by the fact that the range of highest activity is from 4 to 6.5 for acid phosphatase, in addition to the availability of organic matter. Additionally, the medium negative correlation between PA and P (TP and P_2O_5) could be associated with inhibitory effects with increased PT (see section on phosphatase activity).

Finally, we want to highlight that the statistical data showed a low correlation between pH and Psw, and these results are in accordance with those mentioned by Asea et al. (1989) and Salih et al. (1989). The low correlation could be connected to the different mechanisms that are known to solubilize inorganic P (mentioned in the text), indicating that the production of acids plays an important role but is not the only mechanism that could intervene in this solubilization.

Conclusion

The addition of P in the form of PR to cattle manure, poultry manure, and equine manure compost increased the population of P solubilizers, phosphatases activity and ammonium-oxidizing bacteria, but decreased

water-soluble P and nitrate-oxidizing bacteria. Nevertheless, we believe the mineralized organic fertilizer produced by this study could be a source of low-cost macronutrients, minerals, and microorganisms to promote soil health and crop yields.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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