



## Research article

## Effect of biosolids on the nitrogen and phosphorus contents of soil used for sugarcane cultivation

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## ABSTRACT

The application of biosolids improves soil nutrient availability and crop productivity; however, their application needs to be carefully evaluated so as to avoid the risk of contamination. In this study, a 12-month field experiment using a randomized block design with factorial arrangement was conducted to evaluate the effects of biosolids on the nitrogen and phosphorus contents of a sugarcane-cultivated inceptisol. Three types of dewatered biosolids were used: anaerobically digested (B), anaerobically digested and thermally dried (BST), and anaerobically digested and lime-stabilized (BA) biosolids. The results showed that biosolid use increases soil nitrogen content by up to 37% of the total Kjeldahl nitrogen, 42% of  $\text{NO}_3^-$ , 13% of  $\text{NO}_2^-$ , and 32% of  $\text{NH}_4^+$ . Biosolid treatments exceeded the phosphorus requirement for sugarcane cultivation by up to 277% for B, 170% for BST, and 368% for BA. The application of biosolids sufficient to meet crop nitrogen requirements significantly increased soil phosphorus content, suggesting an overdose and low crop response to the available phosphorus. The application of biosolids yielded results similar to those of mineral fertilizers, suggesting their potential use in agriculture.

## 1. Introduction

Sewage sludge is a solid byproduct of wastewater treatment and can be used to produce more stable organic solids, known as biosolids, via treatments such as aerobic or anaerobic digestion, alkaline stabilization, thermal drying, acid oxidation or disinfection, and composting (Collivignarelli et al., 2019). At present, the annual production of biosolids is estimated to be of 100 million tonnes globally, with a projected annual increase of 175 million tonnes by 2050 (Wijesekara et al., 2017).

Biosolids can be used as soil amendments in agriculture and have the potential to replace synthetic fertilizers (Dad et al., 2019; da Mota et al., 2019). Moreover, they can help restore the fertility of agricultural soils deemed infertile owing to the prolonged and indiscriminate use of synthetic inorganic fertilizers (Sharma et al., 2017) and improve infertile and degraded soils at mining sites (Wijesekara et al., 2016).

The characteristics of biosolids vary with respect to the content of organic and inorganic substances, heavy metals, and pathogens (Kumar et al., 2017). These characteristics also depend on the quality of wastewater, type of treatment technology (biological or chemical), and configuration of wastewater treatment plants (WWTPs) because different

types of sludge can be produced (primary, secondary, or mixed sludge) (Raheem et al., 2018; Collivignarelli et al., 2019).

Biosolids are used in agriculture, silviculture, land reclamation, and revegetation because they contain organic matter and essential nutrients for plants (nitrogen and phosphorus) as well as other inorganic elements, such as K, Ca, S, and Mg (Wijesekara et al., 2017; Kumar et al., 2017). The organic and nutrient contents and fertilizer value are influenced by the processes used to stabilize the sewage sludge (Rigby et al., 2016; Dad et al., 2019).

These characteristics make biosolids effective soil amendments as they can improve the physical and nutrient properties of soils and reduce reliance on synthetic fertilizers. These benefits will depend on the quantity of biosolids added to the soil (Sharma et al., 2017).

Biosolids-based soil amendments improve crop productivity and increase the availability of ammonium ( $\text{NH}_4^+$ ), nitrites ( $\text{NO}_2^-$ ), and nitrates ( $\text{NO}_3^-$ ) in the soil due to the mineralization of organic nitrogen (Wang et al., 2017; Wijesekara et al., 2017; Price et al., 2015). Phosphorus is an essential nutrient for plant development; its dynamics in biosolids-amended soils indicate that phosphorus interacts with other nutrients, such as carbon and nitrogen (Torri et al., 2017).

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Although biosolids are highly enriched with nutrients, they might contain pollutants, such as heavy metals, limiting their usage in croplands; therefore, it is necessary to consider the environmental effects of their application in various agroecosystems (Sharma et al., 2017; Stacey et al., 2019). Biosolids may be an important source of nutrients for crops; however, nitrogen may be lost due to leaching, surface runoff, and gaseous emissions caused by ammonia volatilization and denitrification. Leaching of  $\text{NO}_3^-$  into the groundwater is a significant route of nitrogen loss from agricultural systems and a major cause of surface and groundwater contamination because of the low anion-retaining capacity of most soils (Rigby et al., 2016).

Processes such as thermal drying, lime treatment, and composting promote  $\text{NO}_3^-$  loss via increased temperature, pH, and mechanical agitation, respectively (Rigby et al., 2016); these processes also result in loss of ammonium (Silva-Leal et al., 2013b). Phosphorus leaching from biosolids-amended soils is minimal. However, the risk of soluble inorganic phosphorus transport via surface runoff after land application of biosolids is a major concern (Torri et al., 2017).

According to Companhia Ambiental do Estado de São Paulo ((CETESB 1999)) and the United States Environmental Protection Agency (USEPA) (1994), the application rate of biosolids should be determined based on the nitrogen requirements. However, the relatively low nitrogen-to-phosphorus ratio and continuous application of nitrogen-based biosolids have led to the significant overapplication of phosphorus as well as some heavy metals (As, Cd, Cu, Ni, Pb, and Zn). This can modify soil phosphorus dynamics and significantly increase vertical phosphorus transport. As a result, it raises the potential risk of phosphorus release to surface waters via runoff and groundwater contamination by leaching (Sukkariyah et al., 2007; Li et al., 2012; Nogueira et al., 2013; Torri et al., 2017; Wang et al., 2017).

Therefore, it is important to quantify the nutrient release properties of biosolids or any other organic product aimed at replacing a mineral fertilizer. Low nutrient application may result in nutritional deficiencies in crops with subsequent economic loss, whereas its overapplication may

Protection Agency and Colombian regulations (Silva-Leal et al., 2013a; Parra-Orobio et al., 2018; Parra-Orobio, 2020).

## 2. Materials and methods

### 2.1. Study area and experimental units

The experiment was performed in a 0.5-ha lot ( $3^\circ 28' 13.3''\text{N}$   $76^\circ 28' 38.7''\text{W}$ ; 967 m above sea level) located in the city of Cali, Valle del Cauca (Figure 1). At this site, the average temperature is  $24^\circ\text{C}$ , the slope of the land is  $<1\%$ , and the soil is a Vertic Endoaquept with a clay-like texture.

To provide the optimal conditions for seed germination, soil samples were prepared according to the method of Rodríguez and Daza (1995) by uprooting (destruction and incorporation of grass waste into the soil), subsoiling (soil fragmentation up to a depth of 60 cm), plowing (soil fracturing and turning up to a depth of 30 cm), raking (breaking clods to improve contact between seed and soil), and furrowing (distribution of furrows where seeds are sowed).

Each experimental unit was  $6 \times 20$  m with three internal furrows for sowing sugarcane seeds. The CC 8592 sugarcane variety was selected because it is the most commonly used in sugarcane mills in Valle del Cauca (CENICANA, 2002); the stem was portioned to obtain 60-cm long seeds.

### 2.2. Treatments

The following dewatered biosolids were used: (i) anaerobically digested (B), (ii) anaerobically digested and thermally dried at  $60^\circ\text{C}$  for 12.58 h (BST), and (iii) anaerobically digested and lime-stabilized with 9% quicklime for 13 days (BA).

The dose of biosolids applied to the experimental units was calculated based on initial soil characterization, nitrogen dose requirements recommended for sugarcane ( $100\text{ kg ha}^{-1}$ ), and nitrogen availability in biosolids, as indicated in Eq. (1) (CETESB, 1999).

$$\text{Biosolid application dose} = \left( \frac{\text{kg}}{\text{ha}} \right) = \frac{\text{Nitrogen dose recommended for cultivation} \left( \frac{\text{kg}}{\text{ha}} \right)}{\text{Nitrogen availability in biosolids} \left( \frac{\text{kg}}{\text{t}} \right)} \quad (1)$$

negatively affect the environment owing to the lixiviation of nutrients accumulated by surface runoff or erosion and accumulation of heavy metals (Rigby et al., 2016; Dad et al., 2019).

This research was developed in a sugarcane (*Saccharum officinarum* L., Poaceae) cultivation, which occurs in 91 countries. In Colombia, the production of sugar/hectare (ha) is 16.2 tonnes, cultivated in an area of 243,232 ha mainly distributed in five Colombian regions (Valle del Cauca, Cauca, Caldas, Risaralda and Quindío), with Valle del Cauca being the main producer (ASOCANA, 2018).

This study aimed to evaluate the effects of biosolids on nitrogen and phosphorus content of soils used to cultivate sugarcane. The study was conducted on an inceptisol of the suborder Aquepts, great group Endoaquepts, and subgroup Vertic Endoaquept. In Valle del Cauca, this soil type covers a cultivated sugarcane area of nearly 9,777 ha (Carbonell et al., 2006).

The biosolids used in this study were produced at the Municipal Wastewater Treatment Plant from Cali, Colombia. Previous studies based on regular monitoring have not shown the restricted reuse of heavy metals, with low concentrations of As, Cd, Cr, Cu, Hg, Ni, Ag, Pb, B, and Zn, thereby complying with standards of the U.S. Environmental

Nitrogen availability in biosolids (ND) was calculated for surface application assuming a loss of 50%  $\text{NH}_4^+$  as per USEPA (2000) (Equation 2). Additionally, the mineralization constant determined by Silva-Leal et al. (2013b) was calculated for the types of biosolids evaluated in this study.

$$\text{ND} = \left( \frac{\text{K}_{\text{min}}}{100} \right) * (\text{TKN} - \text{NH}_4^+) + 0.5(\text{NH}_4^+) + (\text{NO}_3^- + \text{NO}_2^-) \quad (2)$$

where ND = nitrogen availability in biosolids ( $\text{mg kg}^{-1}$ ),  $\text{K}_{\text{min}}$  = mineralization constant for the type of biosolid, TKN = total Kjeldahl nitrogen of biosolids ( $\text{mg kg}^{-1}$ ),  $\text{NH}_4^+$  = ammonium nitrogen ( $\text{mg kg}^{-1}$ ),  $\text{NO}_3^-$  = nitrates, and  $\text{NO}_2^-$  = nitrites.

Two biosolid application rates based on the nitrogen (N) requirements of sugarcane were evaluated: 1N corresponds to the nitrogen requirement of sugarcane ( $100\text{ kg ha}^{-1}$ ) (Quintero, 1993) and 2N was twice this requirement ( $200\text{ kg ha}^{-1}$ ), as defined by Vieira et al. (2005). Eight treatments were evaluated; these treatments are described in

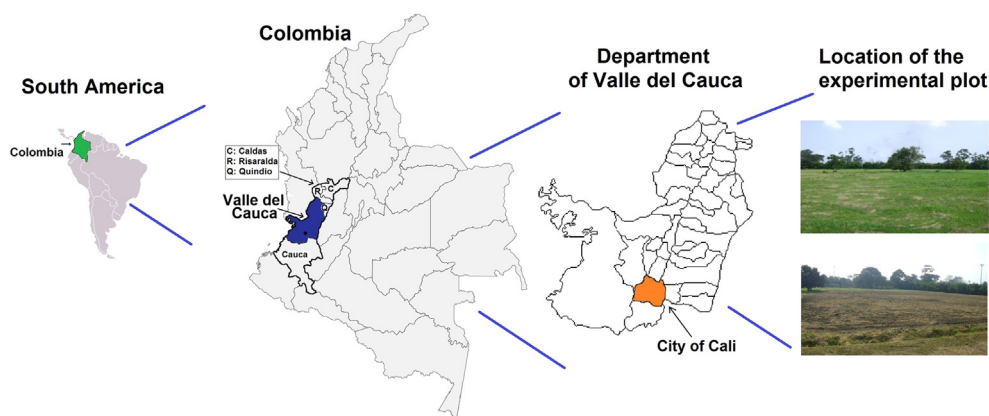


Figure 1. Location of the experimental plot in the city of Cali, Colombia.

**Table 1.** The doses of the biosolids across treatments were calculated based on Eqs. (1) and (2).

A completely randomized block design using factorial arrangement with two factors [biosolid application rates based on nitrogen requirement of sugarcane (1N and 2N) and biosolid type] was used. All treatments were randomly distributed in the study area. Each treatment contained one experimental unit with a 2 m separation between treatment areas within each block and a 6 m separation between blocks to avoid border effects. Replication was performed to ensure the accuracy of statistical analyses, which required 16 experimental units (Figure 2).

The chemical characteristics of the soil and biosolids were determined as follows: pH was measured in deionized water at the sludge:water ratio of 1:2 (Quintero, 1993); organic carbon (Walkley and Black, 1934), TKN (Kjeldahl, 1883),  $\text{NH}_4^+$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ , and P (Bray and Kurtz, 1945) were also measured.

Soil samples were collected from 20 random points distributed across the plot at a depth of 10–20 cm (Quintero, 1993). The composite samples of B, BST, and BA were collected at the WWTP in Cañaveralejo, Cali, Colombia. Previous studies have shown that these biosolids complied with the Environmental Protection Agency and Colombian regulations, and they do not have restrictions in terms of heavy metal contents (USEPA, 1993; Silva et al., 2013a; Parra-Orobio, 2020).

### 2.3. Sowing and monitoring

Before the seeds were sown, the biosolids were applied along the bottom of the furrows at an approximate depth of 15 cm. Mineral fertilizer was applied a month after germination. Sowing was manually

performed by depositing the seeds at the bottom of the furrows, followed by overlapping and covering them with a 10-cm layer of soil.

The dose of the mineral fertilizer was  $217.4 \text{ kg ha}^{-1}$  for urea ( $\text{CH}_4\text{N}_2\text{O}$ ) and  $97.8 \text{ kg ha}^{-1}$  for triple superphosphate ( $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ ); these values were defined based on initial soil characterization and using conventional fertilization for this type of crop in Valle del Cauca (Quintero, 1993).

For crop monitoring, soil samples were collected from the top 20 cm at 10 random sites distributed across each experimental unit (Quintero 1993) at 4, 10, and 12 months after sowing. Further, TKN (Kjeldahl, 1883),  $\text{N-NO}_3^-$ ,  $\text{N-NO}_2^-$ ,  $\text{N-NH}_4^+$  (USDA, 2004), and phosphorus contents were determined (Bray and Kurtz, 1945).

### 2.4. Statistical analysis

Descriptive statistics and analysis of variance were performed using a subsampling component and a significance level of 95% ( $p < 0.05$ ) for the evaluated variables. R version 2.15.0 was used to determine the differences in means using the Tukey's test ( $p < 0.05$ ). Statistical results are expressed as F distribution ( $f$ ) and  $p$ -value ( $p$ ).

## 3. Results and discussion

### 3.1. Chemical characteristics of soil samples and biosolids

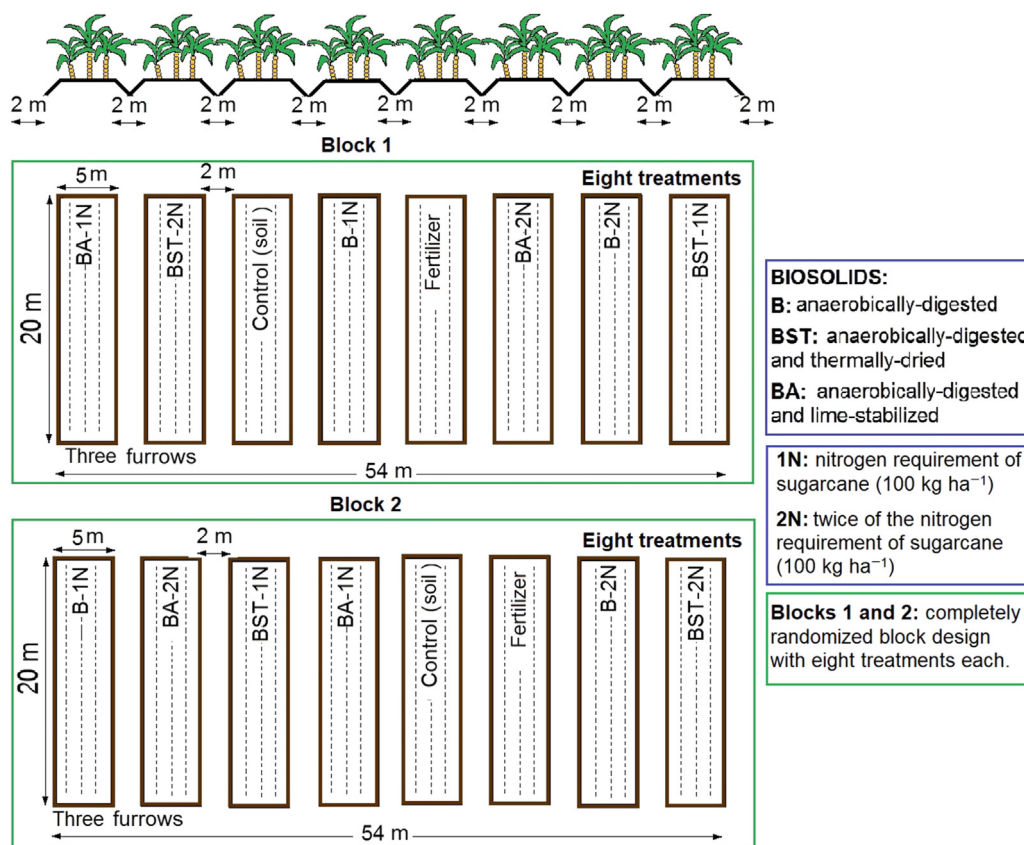
The chemical characteristics of soil and biosolids before sowing are summarized in Table 2. All biosolids (B, BST, and BA) had higher organic carbon and nutrient (nitrogen and phosphorus) content than

**Table 1.** Description of treatments, mineralization constant, and biosolid application rates across treatments.

Treatment	Acronym	Description	Mineralization constant* (%)	Biosolids application ( $\text{t ha}^{-1}$ )
Control treatment	-	Soil only	-	0
Mineral fertilizer	-	Nitrogen (as urea, 46% $\text{CH}_4\text{N}_2\text{O}$ ) and phosphorus (as triple superphosphate, 46% $\text{P}_2\text{O}_5$ )	-	0
Anaerobically digested biosolids (B)	B-1N	Amount of nitrogen in B biosolids based on 1N	33	11.6
	B-2N	Amount of nitrogen in B biosolids based on 2N		23.2
Anaerobically digested and thermally dried biosolids (BST)	BST-1N	Amount of nitrogen in BST biosolids based on 1N	45.7	8.5
	BST-2N	Amount of nitrogen in BST biosolids based on 2N		16.9
Anaerobically digested and lime-stabilized biosolids (BA)	BA-1N	Amount of nitrogen in BA biosolids based on 1N	26	21.1
	BA-2N	Amount of nitrogen in BA biosolids based on 2N		42.2

1N: nitrogen requirement of sugarcane ( $100 \text{ kg ha}^{-1}$ ); 2N: twice the nitrogen requirement of sugarcane ( $200 \text{ kg ha}^{-1}$ ).

\* Values are defined by Silva-Leal et al. (2013b).



**Figure 2.** Scheme of the experimental setup. Randomized block design using factorial arrangement with two factors (biosolid application rates and biosolids type). The biosolid application rates were based on nitrogen requirement of sugarcane (1N: 100 kg ha<sup>-1</sup> and 2N: 200 kg ha<sup>-1</sup>).

**Table 2.** Soil and biosolids chemical characteristics before sowing.

Variable	Soil	B biosolids	BST biosolids	BA biosolids
pH	7.4	7.7	7.8	12.1
Organic carbon (g kg <sup>-1</sup> )	6.8	243.1	257.4	218.2
Total Kjeldahl nitrogen (mg kg <sup>-1</sup> )	1592	25000	25800	17970
N-NH <sub>4</sub> <sup>+</sup> (mg kg <sup>-1</sup> )	8.1	1824	1130	134
N-NO <sub>3</sub> <sup>-</sup> (mg kg <sup>-1</sup> )	4.4	33.8	17.8	34.5
Phosphorus (mg kg <sup>-1</sup> )	7600	14500	14300	9800

B: anaerobically digested biosolids; BST: anaerobically digested and thermally dried biosolids; BA: anaerobically digested and lime-stabilized biosolids.

soil, reaching values that are up to 38 times higher in organic carbon, 16 times in TKN, and 2 times in phosphorus. This supports the potential use of biosolids as a fertilizer or soil amendment (Collivignarelli et al., 2019).

BA biosolids has a basic pH because quicklime is used during chemical stabilization, which tends to benefit acidic soils. However, BA biosolids also increase the buffer capacity of the soil (Flores-Márquez et al., 2010), thereby reducing soil pH and salinity to levels similar to or below

**Table 3.** Variation in heavy metal content in biosolids from WWTP in Cañaveralejo, Cali, Colombia.

Variable	Units	Value	Regulatory Limits	
			USEPA <sup>1</sup>	Colombia <sup>2</sup>
Cadmium	mg kg <sup>-1</sup>	0.48	85	8
Chromium	mg kg <sup>-1</sup>	71.2	3000	1000
Copper	mg kg <sup>-1</sup>	199.75	4300	1000
Nickel	mg kg <sup>-1</sup>	69.3	420	80
Lead	mg kg <sup>-1</sup>	34.1	840	300
Zinc	mg kg <sup>-1</sup>	958.19	7500	2000

<sup>1</sup> USEPA, 1993.

<sup>2</sup> Minvienda (2014).

those of unamended soils (Mendoza et al., 2006). Therefore, multiple additions are recommended for acidic soils (Price et al., 2015).

The soil used in this study had a lower organic carbon content than expected when compared with that in soils from the Valle del Cauca region (20–40 g kg<sup>-1</sup> organic carbon) (Quintero, 1993) possibly because of the progressive loss of fertility in this region, with 32% of soils having less than 20 g kg<sup>-1</sup> organic carbon (Luna, 2006).

Dad et al. (2019) used seven types of biosolids and suggested that these biosolids have value as fertilizers and can easily replace synthetic fertilizer for agricultural use. Therefore the characteristics of the soils used in this study would likely be improved using biosolids as soil amendments in order to increase organic carbon content and ensure optimal growth of sugarcane (Luna, 2006; Xue et al., 2015; da Mota et al., 2019).

The chemical characteristics of the biosolids used here are similar to those of biosolids reported in other studies—e.g., organic matter content between 242 and 910 g kg<sup>-1</sup>, TKN content between 15,000 and 68,000 mg kg<sup>-1</sup>, and P content between 1,000 and 36,000 mg kg<sup>-1</sup> (Vieira et al., 2005; Boeira and Maximiliano, 2009; Rigby et al., 2016; Kumar et al., 2017; Dad et al., 2019; Collivignarelli et al., 2019). Notably, thermal drying of biosolids does not affect their chemical characteristics (Smith and Durham, 2002; Ramírez et al., 2008; Tarrasón et al., 2008). Both BST and B biosolids have similar chemical characteristics.

BA biosolids had lower nitrogen content than B and BST biosolids, which is similar to the results of Mendoza et al. (2006) and Rigby et al. (2016). The pH probably increased because lime stabilization promotes NH<sub>3</sub> loss (Méndez et al., 2002; Rigby et al., 2016), which results in a loss of 28% and 30% of total nitrogen compared with the loss in B and BST biosolids, respectively (Czechowski and Marcinkowski, 2006; Plachá et al., 2008).

Although biosolids can be effectively recycled and used as soil amendments for agricultural crops because they contain several important micro- and macronutrients, they should only be used in agriculture if toxicity from heavy metals that accumulate in soil can be avoided (Dad et al., 2019). Previous studies have shown that biosolids used in this experiment do not have restrictions in terms of heavy metal content

(Silva et al., 2013a). Although the objective of this research did not include the analysis of heavy metals, some metals were measured in order to verify their concentrations (see Table 3). The reported values are low compared with the USEPA regulatory limits and Colombian regulations.

### 3.2. Effects of biosolid application on soil nitrogen and phosphorus contents

Table 4 shows the variation in the nitrogen forms (TKN, NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, and NH<sub>4</sub><sup>+</sup>) during cultivation. Figure 3 shows the variations in TKN content, the predominant form of nitrogen across treatments.

Four months after sowing, biosolids were associated with an increase in TKN content in the soil (37%). B-1N (37%), BST-2N (34%), and B-2N (30%) showed similar and even higher percentages, when compared with the mineral fertilizer (30%). Notably, after biosolid application, the TKN content of soil increased from 1592 mg kg<sup>-1</sup> to approximately 2000 mg kg<sup>-1</sup>, reaching the minimum nitrogen value reported for soils cultivated with sugarcane in the Valle del Cauca region (Quintero, 1993).

BA-1N (2%) and BST-1N (8%), had the lowest TKN content; these results are consistent with those reported by Vieira et al. (2005) who observed an increase in TKN content within the first 15 days of corn cultivation, ranging from 2%–44% for doses up to 8 times the nitrogen requirement.

Analysis of variance revealed no significant differences in TKN content in the treatments over the treatment periods (months): 4 ( $f = 2.45, p = 0.129$ ), 10 ( $f = 0.48, p = 0.819$ ), and 12 ( $f = 1.60, p = 0.274$ ). Moreover, there were no significant differences in 1N and 2N treatments (month 4:  $f = 3.39, p = 0.1247$ ; month 10:  $f = 0.0701, p = 0.8018$ ; and month 12:  $f = 2.214, p = 0.196$ ).

Four months after sowing, mineral fertilizer treatment showed 621% of NO<sub>3</sub><sup>-</sup>, 76% of NO<sub>2</sub><sup>-</sup>, and 76% of NH<sub>4</sub><sup>+</sup>; these values are higher than those for biosolids. B1-N and BST-2N achieved the highest content of these nitrogen forms across biosolids compared with untreated soil, reaching values of up to 42% for NO<sub>3</sub><sup>-</sup>, 13% for NO<sub>2</sub><sup>-</sup>, and 32% for NH<sub>4</sub><sup>+</sup>. The other biosolid treatments had a content lower than or equal to that in soil. Ten

**Table 4.** TKN, N-NO<sub>3</sub><sup>-</sup>, N-NO<sub>2</sub><sup>-</sup>, and N-NH<sub>4</sub><sup>+</sup> content (mean ± standard deviation) across treatments.

Treatment	Month	TKN mg kg <sup>-1</sup>	N-NO <sub>3</sub> <sup>-</sup> mg kg <sup>-1</sup>	N-NO <sub>2</sub> <sup>-</sup> mg kg <sup>-1</sup>	N-NH <sub>4</sub> <sup>+</sup> mg kg <sup>-1</sup>
Control (soil)	4	1854.4 ± 379.8	13.22 ± 1.34	0.46 ± 0.17	12.30 ± 0.64
Mineral fertilizer	4	2407.6 ± 144.6	95.39 ± 6.33	0.81 ± 0.51	21.66 ± 0.91
B-1N	4	2542.1 ± 261.3	18.69 ± 12.01	0.52 ± 0.28	14.30 ± 2.34
B-2N	4	2411.5 ± 66.37	17.29 ± 18.85	0.27 ± 0.17	14.67 ± 2.73
BST-1N	4	2007.4 ± 298.5	12.83 ± 0.11	0.44 ± 0.17	13.26 ± 0.22
BST-2N	4	2476.4 ± 387.3	18.77 ± 6.55	0.33 ± 0.20	16.23 ± 5.03
BA-1N	4	1889.0 ± 333.4	10.60 ± 3.08	0.30 ± 0.01	12.82 ± 2.55
BA-2N	4	2332.6 ± 40.27	9.88 ± 0.45	0.28 ± 0.05	14.50 ± 4.25
Control (soil)	10	1672.7 ± 160.8	6.07 ± 1.09	0.10 ± 0.14	0.94 ± 0.2
Mineral fertilizer	10	1949.5 ± 145.3	6.54 ± 0.76	0.02 ± 0.02	0.62 ± 0.48
B-1N	10	2154.4 ± 31.6	6.08 ± 1.95	0.02 ± 0.01	0.32 ± 0.01
B-2N	10	1977.9 ± 440.2	5.89 ± 0.38	0.03 ± 0.02	0.59 ± 0.60
BST-1N	10	1951.3 ± 299.9	6.92 ± 2.16	0.06 ± 0.07	0.51 ± 0.21
BST-2N	10	1967.7 ± 752.5	7.72 ± 0.51	0.02 ± 0.01	0.97 ± 0.41
BA-1N	10	1564.5 ± 380.8	6.65 ± 0.42	0.15 ± 0.19	1.13 ± 0.16
BA-2N	10	1924.9 ± 12.33	5.36 ± 0.18	0.11 ± 0.09	0.61 ± 0.58
Control (soil)	12	1362.8 ± 412.1	31.81 ± 11.43	0.28 ± 0.04	7.84 ± 2.56
Mineral fertilizer	12	1774.6 ± 132.5	24.24 ± 0.14	4.07 ± 5.23	10.16 ± 6.87
B-1N	12	1619.5 ± 242.9	22.10 ± 5.58	5.67 ± 6.24	9.52 ± 6.83
B-2N	12	1025.2 ± 43.7	21.03 ± 6.45	0.96 ± 0.63	2.62 ± 1.48
BST-1N	12	1619.2 ± 670.5	26.34 ± 5.63	5.91 ± 8.01	4.56 ± 0.47
BST-2N	12	830.9 ± 270.7	18.82 ± 1.29	0.35 ± 0.16	2.92 ± 1.25
BA-1N	12	983.9 ± 327.6	14.23 ± 8.47	0.30 ± 0.02	4.31 ± 0.81
BA-2N	12	1371.5 ± 409.1	23.12 ± 1.67	0.94 ± 0.36	6.28 ± 1.77



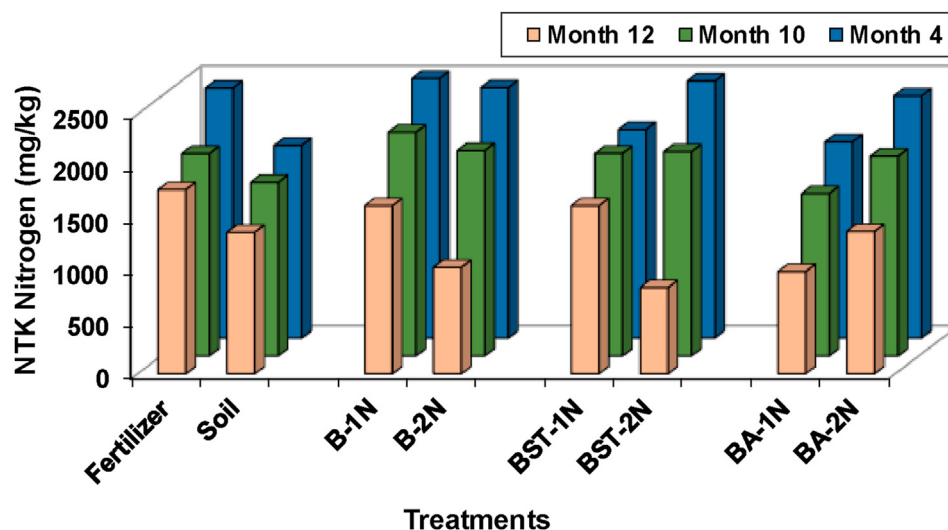


Figure 3. Variation in TKN content across treatments during the cultivation period.

and twelve months after sowing, the content of these nitrogen forms drastically decreased following mineral fertilizer treatment, with a value similar to that observed in other treatments.

Four months after sowing, significant differences were observed in the  $\text{NO}_3^-$  content following mineral fertilizer treatment compared with that observed following other treatments ( $f = 23.71$ ,  $p = 0.00022$ ). Such differences were observed because the fertilizer was applied 1 month after germination, showing a 621% increase in  $\text{NO}_3^-$  content. No significant differences either in the remaining months (month 10:  $f = 0.73$ ,  $p = 0.651$  and month 12:  $f = 1.21$ ,  $p = 0.402$ ) or nitrogen dose (month 4:  $f = 0.067$ ,  $p = 0.805$ ; month 10:  $f = 0.0983$ ,  $p = 0.766$ ; and month 12:  $f = 0.0009$ ,  $p = 0.9773$ ) were observed.

$\text{NH}_4^+$  content considerably decreased by the 10<sup>th</sup> month across treatments because it is more readily available and easily assimilated by plants following biosolid application (Sullivan et al., 2015). Although BA biosolids showed the lowest  $\text{NH}_4^+$  content ( $134 \text{ mg kg}^{-1}$ ) at the beginning of the study, this condition did not influence the behavior of  $\text{NH}_4^+$  in the soil, which was present at concentrations similar to those observed in other treatments throughout the cultivation period.

The results were verified via analysis of variance, which indicated that no significant differences were observed among the treatments (month 4:  $f = 2.70$ ,  $p = 0.106$ ; month 10:  $f = 0.941$ ,  $p = 0.531$ ; and month 12:  $f = 1.1545$ ,  $p = 0.4273$ ). Moreover, no differences were observed between the two 1N and 2N treatments (month 4:  $f = 1.127$ ,  $p = 0.337$ ; month 10:  $f = 0.0934$ ,  $p = 0.7722$ ; and month 12:  $f = 1.3494$ ,  $p = 0.2978$ ).

There was no evidence that thermal drying or alkaline treatment of BST and BA biosolids affected nitrogen mineralization in the soil; both these treatments yielded results similar to those observed for B biosolids. This effect of BST has been reported previously (Smith and Durham, 2002; Ramírez et al., 2008; Tarrasón et al., 2008). For BA, with an acidic-to-slightly alkaline pH range (<8), increasing soil pH can increase N mineralization rate in biosolids-amended soil (Rigby et al., 2016). However, Carneiro et al. (2005) found that biosolids treated with CaO had decreased nitrogen mineralization, thereby increasing loss due to volatilization or lixiviation.

Notably, an increase in soil pH was not caused by the application of BA biosolids. Clearly, B, BST, and BA biosolids enhanced soil nutrient availability and behaved in a manner similar to that of mineral fertilizer treatment throughout the cultivation period, except for  $\text{NO}_3^-$  content in month 4. Regardless of the nitrogen dose applied (1N and 2N), B and BST biosolids yielded higher nitrogen content than BA biosolids.

Although no significant differences were observed between the 1N and 2N treatments, the amount of applied nitrogen should never exceed

that required by the crop because it can surpass the absorption capacity of plant roots. This may lead to nitrogen loss and groundwater pollution, mostly due to  $\text{NO}_3^-$ , which is the most soluble nitrogen form and has the highest mobility in soil (USEPA, 1995; Li et al., 2012; Nogueira et al., 2013; Rigby et al., 2016; Wang et al., 2017).

Figure 4 shows the variation in phosphorus content [mean  $\pm$  standard deviation ( $\sigma$ )] throughout the cultivation period. All biosolid treatments increased the phosphorus content of the soil from 11% to 198% (month 4), from 54% to 260% (month 10), and from 57% to 277% (month 12), with B biosolids contributing the most to the increase in phosphorus content. Mineral fertilizer treatment showed a higher phosphorus content than biosolid treatment, at approximately 312% (month 4), 258% (month 10), and 543% (month 12).

After recalculating the application rate of biosolids based on the phosphorus requirement of sugarcane and the amount available from each biosolid, the required doses were much lower than those based on nitrogen requirements ( $3.10 \text{ t ha}^{-1}$  for B biosolids,  $3.14 \text{ t ha}^{-1}$  for BST biosolids, and  $4.5 \text{ t ha}^{-1}$  for BA biosolids). This implies a significant oversupply of P, at 274% for B biosolids, 170% for BST biosolids, and 368% for BA biosolids.

The applied dose of biosolids exceeded the phosphorus requirement for sugarcane, suggesting that dose estimation based on nitrogen requirement results in phosphorus overdose in the soil. This potential risk, which may significantly increase vertical phosphorus movement, has also been previously reported (Sepúlveda et al., 2011; Li et al., 2012; Torri et al., 2017).

Sepúlveda et al. (2011) found that the overapplication of biosolids (1.4 years) increased the available phosphorus content by 180% when compared to a biosolids-unamended inceptisol. Although phosphorus is a vital macronutrient in sugarcane physiology (Quintero, 1993), our results showed that probably phosphorus assimilation by sugarcane was low, reflected by the high phosphorus content of the soil. In addition, the application of high doses may decrease the phosphorus fixation capacity of the soil due to the formation of organic acids during biosolid decomposition, which blocks the adsorption sites of phosphorus in the solid phase (Munhoz and Berton, 2006). It is recommended to contrast these results with the measurement of phosphorus content in the plant.

No significant differences in phosphorus content in the soil were observed across treatments (month 4:  $f = 1.50$ ,  $p = 0.30$ ; month 10:  $f = 1.11$ ,  $p = 0.445$ ; and month 12:  $f = 1.97$ ,  $p = 0.194$ ). Similarly, the applied doses of biosolids were not significant for this variable (month 4:  $f = 3.52$ ,  $p = 0.119$ ; month 10:  $f = 0.1477$ ,  $p = 0.716$ ; and month 12:  $f = 0.276$ ,  $p = 0.621$ ).

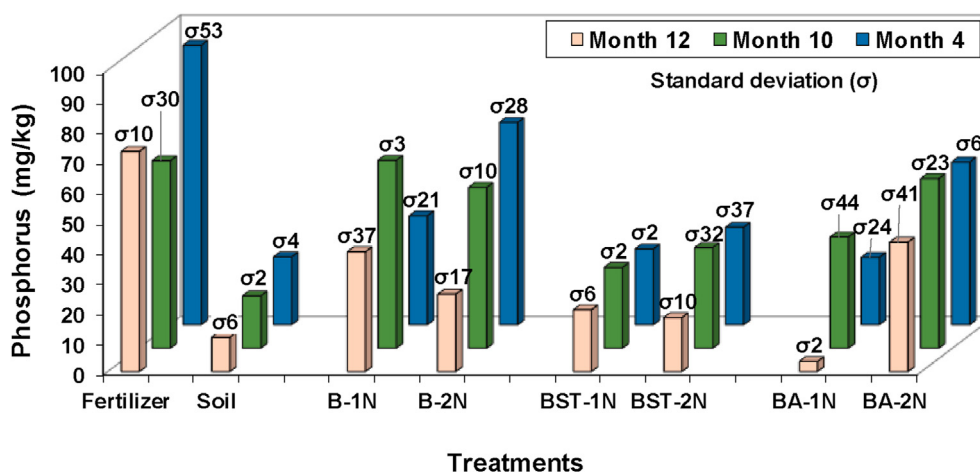


Figure 4. Variation in phosphorus content [mean  $\pm$  standard deviation ( $\sigma$ )] across treatments throughout the cultivation period.

Long-term biosolids-based soil amendments can strongly impact microbial communities that are not detected in a short-term study (Stacey et al., 2019). Wang et al. (2017) assessed the effect of biosolid application on pine cultivation over a 19-year period and showed a low crop response to available phosphorus possibly because of phosphorus immobilization in the soil or low absorption efficiency of roots, controlled by mycorrhizal association. Therefore, extended biosolid application can substantially change the structure and function of the microbial groups present in the soil, which are responsible for the variations in nitrogen and phosphorus availabilities.

Our results showed a potential application of biosolids to agriculture owing to their nitrogen and P content, with effects similar to mineral fertilizers. Thus, synthetic fertilizers can be replaced by biosolids (Sharma et al., 2017; Dad et al., 2019). However further studies are needed to ensure that there is no risk of accumulating undesirable chemicals in the soil.

Because extended and successive rounds of biosolid application may substantially increase nutrient availability (particularly phosphorus) and affect microbial content (Trannin et al., 2007; Wang et al., 2017), it is advisable to estimate the doses of biosolids as a function of phosphorus requirements in order to minimize the risks of environmental pollution and eutrophication. Furthermore, we suggest performing additional studies to quantify the nitrogen content that may be lost due to lixiviation, as observed with mineral fertilizer treatment at month 4. Finally, although the biosolids used in this research were not limited by their heavy metal content, other micropollutants and should also be considered. Additionally, we recommended to evaluate the effects of the application of biosolids on plant biomass.

#### 4. Conclusions

Biosolid application increased the content of nitrogen and phosphorus of the soil throughout the study period. The nitrogen and phosphorus contents were higher following the application of B and BST biosolids than after the application of BA biosolids.

No significant differences between mineral fertilizer treatment and biosolid treatments were observed and efficient sugarcane growth and productivity were achieved. Therefore, biosolids can be potentially used as a replacement for synthetic fertilizers in sugarcane cultivation.

The application rate of biosolids based on N requirements significantly increased the phosphorus content of the soil from 11% to 277%, indicating an overdose and low crop response to the available phosphorus. This was also observed for mineral fertilizer treatment. Therefore, we recommend calculating the application rate of biosolids based on phosphorus requirements, thereby reducing potential water and groundwater pollution.

#### Declarations

##### Author contribution statement

Jorge Antonio Silva-Leal: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Andrea Pérez-Vidal: Performed the experiments; Wrote the paper.

Patricia Torres-Lozada: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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##### Data availability statement

Data included in article/supplementary material/referenced in article.

##### Declaration of interests statement

The authors declare no conflict of interest.

##### Additional information

No additional information is available for this paper.

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