

Short-term amendment of biosolid on agricultural soil: effects on C and N mineralization and microbial activity

Enmienda a corto plazo de un suelo agrícola con biosólidos: efectos sobre la mineralización de C-N y la actividad microbiana

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Abstract

Adding biosolids to agricultural soils can improve its quality through increased storage of C and N. A study to analyze the short-term impact of the addition of biosolids on the release of nutrients and the microbial activity in agricultural soil was carried out. The microbial biomass C (MB-C), urease activity (UA), and mineralization of N and C at different application rates of biosolids were evaluated (0 mg, 100mg and 200 mg of N-NH₄⁺ kg⁻¹). In addition, a biosolid-only treatment was tested. It was observed an increase in C and N mineralization, NH₃ volatilization, and MB-C content, according to the application rate of biosolids. In biosolid treatments, UA increased 100% on average in the first seven days of incubation. These results suggest that the nutrient content in the soil is improved and microbial activity is positively stimulated.

Keywords: Urease activity; microbial biomass; CO₂ emissions; nitrogen available.

Resumen

La adición de biosólidos a suelos agrícolas puede ayudar a mejorar la calidad de dichos suelos mediante un mayor almacenamiento de C y N. Se realizó un estudio para analizar el impacto a corto plazo de la adición de biosólidos en la liberación de nutrientes y la actividad microbiana en el suelo. Se evaluó el C de la biomasa microbiana (MB-C), la actividad ureasa (UA) y la mineralización de N y C a diferentes dosis de aplicación de biosólidos (0 mg, 100 mg y 200 mg de N-NH₄⁺ kg⁻¹). Además, se probó un tratamiento solo con biosólidos. Se observó un aumento en la mineralización de C y N, volatilización de NH₃ y contenido de MB-C, conforme a la tasa de aplicación. La UA aumentó en promedio 100% en los primeros siete días de incubación para los tratamientos de biosólidos. Estos resultados sugieren una mejora en el contenido de nutrientes y una estimulación positiva de la actividad microbiana.

Palabras clave: Actividad ureasa; biomasa microbiana; emisión de CO₂; nitrógeno disponible.

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Introduction

Biosolids are sludge generated during the treatment of wastewater and stabilized physically, chemically, or biologically, and are therefore susceptible to reuse (Sharma *et al.*, 2017). It is estimated that the United States of America and Europe produce approximately 7.2 and 10 million tons per year, respectively (Lloret *et al.*, 2016), while Mexico generates 232 thousand tons per year (Gutiérrez–Avedoy *et al.*, 2012). The State of Guanajuato, in Mexico, produces 31 400 t per year, from which 2380 t are estimated to be produced in the municipality of Celaya, Guanajuato (Universidad Nacional Autónoma de México [UNAM], 2014).

The application of biosolids to soils is a common practice to maintain or improve microbial and enzymatic activity and soil quality, especially in soils that are low or deficient in organic matter content (Rigby *et al.*, 2016). Biosolids are abundant in organic matter content, and micro and macronutrients, making it an economic and sustainable substitute instead of inorganic fertilizers (Alvarenga *et al.*, 2016). In addition, soils amended with biosolids improve their physical properties such as porosity, apparent density, aggregate stability, and water retention capacity, having a positive impact on the yield of various crops (Roig *et al.*, 2012). However, the use of biosolids should be periodically evaluated and monitored for the presence of contaminants and nutrient releases (C and N) that may limit their safe disposal to the environment or their use in agricultural systems (Clarke & Cummins, 2015).

On the other hand, over the years, the biological and biochemical properties of soil have gained importance in assessing the sustainability of ecosystems (Singh *et al.*, 2015). Soil microorganisms play an important role in organic matter mineralization, nutrient recycling, and energy flow processes in biogeochemical cycles (Ferreira *et al.*, 2016). Similarly, soil enzymes are of importance in soil productivity and fertility because of their constant production, accumulation, inactivation, or degradation in the soil (Burns *et al.*, 2013). Most biological processes in the soil depend on enzymatic activity, the release of nutrients in forms available to plants and microorganisms, and the breakdown of organic matter (Sharma *et al.*, 2017).

In the same context, urease hydrolyzes urea and amides, producing CO₂ and NH₃. Urease is important because of its relationship to the nitrogen cycle (Celis *et al.*, 2011; Tabatabai, 1994). According to Azeem *et al.* (2016), increased urease activity in soil is associated with increased microbial biomass content, which coincides with Nannipieri *et al.* (1979), who suggested that urease activity (UA) increases with microbial biomass, since this is an intracellular constituent. However, Adetunji *et al.* (2017) established that a higher UA is associated with higher organic matter content in the soil rather than increase in microbial biomass.

Currently, there are no studies on the effect of the addition of biosolids to agricultural soils in the Bajío region of Mexico pertaining C and N mineralization and microbial activity. The objective of this study was to evaluate the mineralization of C and N, the microbial biomass carbon (MB-C), and UA as a result of the short-term application of biosolids at different doses.

Materials y Methods

The study was carried out on agricultural land located south of Celaya, Guanajuato, with geographic coordinates 20° 30' 25" N and 100° 52' N.37" W. The soil was sampled in April 2017 and a representative area of 100 m x 100 m was selected. Soil samples were taken using the zigzag method by dividing the area into three equal zones, with 12 sampling points in each zone. At each point a sample of approximately 0.5 kg was collected to form composite samples of 6 kg per zone. The samples were dried, homogenized and sieved using a mesh with a 2-mm hole and then stored at 4 °C until analysis.

The biosolid was sampled in July 2017 at a municipal wastewater treatment plant in the Bajío region of Mexico, located at the geographic coordinates 100° 51' 64" W and 20° 30' 78" N. The biosolids were produced by aerobic digestion processes and dehydrated by means of pressed band filtration systems. The biosolids were taken six times over a four-hour period on the conveyor belt at the outlet of the filter press. Approximately 50 kg in total of the sample on a wet basis (85%) was collected. After that, the sample was taken to the laboratory, dried in the air, sieved through a 2 mm diameter, and stored at 4 °C for further analysis. The analysis of their properties did not exceed the storage time of one week. The biosolids were class "C", which could be used for agricultural and soil improvement purposes. The content of parasites, pathogens and heavy metals in the biosolids was under the permissible limits for their use according to current Mexican regulations (Diario Oficial de la Federación [DOF], 2003).

The results obtained, both in the characterization of the soil and biosolid and in the incubation experiments for the mineralization dynamics, are expressed on a dry basis.

Physicochemical characterization of soil and biosolid

The pH and electric conductivity (EC) were measured in an aqueous extract (1:5 w/v) with the help of a digital pH and conductivity meter (Rhoades, 1996; Thomas, 1996). Soil texture was determined by the Bouyoucos method (Gee & Bauder 1986). Total organic carbon (TOC) was analyzed by oxidation with potassium dichromate according to the Walkley & Black (1934) method and modified by downscaling and colorimetric determination (Alef & Nannipieri, 1995). Total nitrogen (TN) was analyzed by the micro-Kjeldhal system (Bremner, 1996). Inorganic nitrogen (NH_4^+ , NO_3^- , and NO_2^-) was extracted from the samples with a solution of K_2SO_4 0.5 M (1:6 w/v) and colorimetrically determined (Fernández-Luqueño *et al.*, 2016; Keeney & Nelson, 1982). In addition, the C/N ratio was obtained.

Experimental design to evaluate the dynamics of C and N mineralization

The effect of the application of different doses of biosolids on C and N mineralization and microbial activity in laboratory experiments for 56 days was determined. For experimentation, 50 g of soil were weighed into 100 ml glass jars (microcosm). A droplet of deionized water was added to the soil surface with a pipette up to 60% water retention capacity (WHC). The samples were then pre-incubated at 25 °C for 7 days in the dark. After pre-incubation, 50 g of soil were modified with different application rates of biosolids. The application rate of biosolids based on the soil NH_4^+ content was 0 mg kg^{-1} (T0 treatment = 0 Mg ha^{-1}), 100 mg kg^{-1} (T1 treatment, 30 Mg ha^{-1} of biosolids), and 200 mg kg^{-1} (T2 treatment, 60 Mg ha^{-1} of biosolids). In addition, 50 g of biosolids without soil (T3 treatment) were weighed to evaluate the mineralization of organic matter in the biosolid without the influence of soil processes. Each treatment was performed in triplicate.

At the start of incubation, the moisture content was adjusted to 60% of the WHC for each microcosm and placed in 1 L jars. In addition, two vials of 20 ml of 1 M NaOH solution and 2% H_3BO_3 solution were added to each jar to trap the CO_2 emitted and NH_3 volatilized, respectively. The 1 L jars with the microcosms and vials were hermetically sealed and incubated at 25 °C in the dark for 56 days. To maintain aerobic conditions, all the jars were opened for 10 min every 15 days and the vials containing the 1 M NaOH solution were removed to prevent atmospheric CO_2 from being trapped (López-Valdez *et al.*, 2010).

After 0, 1, 3, 7, 14, 28, and 56 days, three 1 L jars were randomly selected for analysis (Fernández-Luqueño *et al.*, 2017). In addition, all the treatments and subsamples of soil were analyzed for MB-C, UA, and inorganic nitrogen content (NH_4^+ , NO_2^- , and NO_3^-) over time.

Determination of physicochemical characteristics in mineralization dynamics

The CO₂ trapped in the NaOH solution vials was determined by volumetric titration with a solution of 1 M HCl and phenolphthalein as an indicator. Prior to titration, 1 ml of 0.5 M BaCl₂ solution was added to precipitate the carbonates. In parallel, three blanks were analyzed in the same way as the treatments for each day of sampling (Rojas-Oropeza *et al.*, 2010). The percentage of mineralized C was determined by calculating the organic C emitted to CO₂ in relation to the organic C added to the biosolids.

The volatilized NH₃ was determined with the titration of H₃BO₃ at 2% with solution of H₂SO₄ 0.1 M (Beltrán-Hernández *et al.*, 2007). The concentrations of NH₄⁺, NO₂⁻, and NO₃⁻ were obtained by extraction from the soil matrix with 0.5 M K₂SO₄ solution and measured colorimetrically (Fernández-Luqueño *et al.*, 2016; Keeney & Nelson, 1982).

The nitrogen available in the biosolids was calculated using the equation reported by Andreoli *et al.* (2001):

$$N_{available} = f_{org}(N_{org}) + f_{vol}(NH_4^+) + NO_3^-$$

where f_{org} = percentage of mineralized biosolids in the studied soil; f_{vol} = percentage of volatilized NH₃ during the incubation period; N_{org} , NH_4^+ , NO_3^- = organic nitrogen, ammonium, and nitrate content, respectively.

Determination of microbial activity in mineralization dynamics

The quantification of MB-C by the substrate-induced respiration method (SIR) was determined. One jar of 100 ml containing 20 g of soil with glucose as substrate at a concentration of 1750 mg kg⁻¹ (dry base) and one vial of 25 ml containing 0.1 M NaOH solution were placed in a 1 L jar and incubated for 4 h at 25 °C. The NaOH solution was titrated with 0.1 M HCl. The MB-C concentration was determined using the equation established by Anderson & Domsch (1978):

$$C_{mic} = 30(B - S) \left[\frac{(K)(22)(1000)}{(1.8295)(SW)(4)} \right]$$

where C_{mic} = Microbial biomass carbon (μg g⁻¹); B = volume used in the titration of the HCl solution for the controls (ml); S = volume used in the titration of the HCl solution for the samples (ml); K = HCl solution concentration (mmol ml⁻¹); SW = sample weight (g); 30 = microbial respiration constant (mg C_{mic} h ml⁻¹ CO₂); 22 = CO₂ absorption factor (1 mmol HCl – 22 mg CO₂); 1000 = Conversion factor (μg C_{mic} at g C_{mic}); 1.8294 = CO₂ density at 0 °C and 101.32 Pa (mg ml⁻¹); and 4 = incubation time (h).

For the determination of UA, 1 g of dry soil was placed in a 100 ml vial and mixed with 2.5 ml of 80 mM urea as substrate. Then, it was shaken and incubated for 2 h at 37 °C. After that, 50 ml of a 1 M KCl solution was added and shaken for 30 min and then filtered out. From the filtrate, 1 ml was taken, and 9 ml of sterile distilled water was added to dilute the extract in a 1:10 ratio. For color development, 5 ml of 5.6% sodium salicylate solution dissolved in 0.1 M NaOH solution and 2 ml of 0.1% sodium dichloroisocyanurate solution were mixed, and the absorbance was measured at 690 nm after 30 min. Each sample was compared to a white one, which was prepared in the same way as the sample, with the only difference that distilled water was added instead of urea. UA was expressed with respect to the NH₄⁺ content in the corresponding treatment (Kandeler & Gerber, 1988).

Statistical analyses

The experimental units were the microcosms, and the experimental design was completely random. Statistical analysis of the data was performed using Minitab Statistical Software. To determine the statistical significance of the differences between the averages of treatments, an ANOVA was performed followed by Tukey's test ($p \leq 0.05$) on the results of the experiment.

Results

The physicochemical characterization of the soil and biosolids used in the experiments are shown in Table 1. The soil presented a pH value of 6.83, classifying it as neutral. The soil presented an EC of 0.67 dS m^{-1} . Soils with EC and pH values greater than 4 dS m^{-1} and 8.5, respectively, reflect salinity problems (DOF, 2000). Thus, the fertility of this soil, which presented lower values than those reported, was not affected by a high content of salts in the soil solution. Furthermore, the mineralization of the biosolids added to the soil was not affected by the presence of salts in the soil. The soil presented 70% clay, 22% sand and the rest of silt, classifying it as a fine textured soil (Shirazi & Boersma, 1984). Fine textured soils can lead to the physical/chemical protection of organic matter or reduce substrate availability, making it inaccessible to microorganisms and enzymes (Cassidy-Duffey *et al.*, 2020). In addition, a decrease in N mineralization has been reported in fine textured soils (Sorensen & Jensen, 1995). The soil had a content of TOC of 55.15 g kg^{-1} , so it is classified as a medium fertility soil, as established in Mexican regulations (DOF, 2000). On the other hand, the TN content (2.10 g kg^{-1}) was found within the parameters that have been reported for agricultural soils by other studies (Zheng *et al.*, 2013). The inorganic N content (sum of NH_4^+ , NO_3^- , NO_2^-) in the soil was $144.59 \text{ mg kg}^{-1}$. The obtained value of inorganic N in the soil is classified as very high for fertility according to Mexican regulations (DOF, 2000). Loss of N has been observed in agricultural soils regardless of the inorganic N content it has (Chen *et al.*, 2014). The C/N ratio controls the development of the soil microbiota as well as the process of mineralization of organic matter (Probert *et al.*, 2005). The C/N ratio in the soil was 26.26, which represents a soil with excess carbon and energy (soils with a C/N ratio > 11.5). Therefore, an organic amendment is required that does not release all its energy or organic matter in the short term, that is, an amendment with a lower C/N ratio than that of the soil. Thus, the release of mineral N and the TOC content in the soil can be controlled to reach C/N ratio values of 8.5 to 11.5, which are considered ideal in agricultural soil (Abbasi *et al.*, 2015).

The biosolids presented a pH of 7.37, which is considered neutral. The EC value was 3.84 dS m^{-1} , which is higher than that presented by the soil. The TOC and TN content were 78.21 g kg^{-1} and 6.88 g kg^{-1} . The C/N ratio was 11.37, so it was expected that, when added to the soil, the N content would increase. Inorganic N content (sum of NH_4^+ , NO_3^- , NO_2^-) of the biosolids was 1.008 g kg^{-1} , which represents an important contribution of mineral N that could be reflected in the volatilization of N as NH_3 . These reported values for the different parameters in biosolids coincide with those reported in other studies (de Melo W *et al.*, 2018; Medina-Herrera *et al.*, 2020).

The pH of the soil and biosolids samples was 6.83 and 7.29, this parameter was not significantly affected by adding the biosolids to the soil at the beginning and end of the experiment ($p \leq 0.05$). However, a downward trend was observed in pH in T1, T2, and T3 treatments, with values of 6.72, 6.52, and 7.15 at the end of incubation. With respect to the EC, the amended treatments with biosolids (T1 and T2) showed a significant increase by 2.40 and 3.10 times ($\text{EC} = 1.64$ and 2.12 dS m^{-1}) from the initial soil value. The T0 and T3 treatments showed no significant changes ($p \leq 0.05$) in the EC with respect to the beginning of the experiment.

Table 1. Physicochemical characteristics of the soil and biosolids used in the experiment.

	Soil	Biosolid
pH	6.83 ± 0.10	7.37 ± 0.06
EC (dS m ⁻¹)	0.67 ± 0.03	3.84 ± 0.02
Texture	Clay	-
TOC (g kg ⁻¹)	55.15 ± 1.76	78.21 ± 2.37
TN (g kg ⁻¹)	2.10 ± 0.77	6.88 ± 0.05
C/N	26.26 ± 2.51	11.37 ± 0.84
NH ₄ ⁺ (mg kg ⁻¹)	120.57 ± 11.99	859.31 ± 13.53
NO ₃ ⁻ (mg kg ⁻¹)	23.13 ± 4.07	129.68 ± 1.72
NO ₂ ⁻ (mg kg ⁻¹)	0.89 ± 0.24	18.52 ± 4.01

Note. The values represent the mean, ± represents the standard deviation ($n = 3$). The results are obtained on a dry basis from the soil and biosolid as appropriate.

Source: Author's own elaboration.

The CO₂ emission presented significant differences ($p \leq 0.05$) among treatments (Figure 1). The CO₂ derived from the mineralization of the biosolids is the CO₂ emission in the T1 and T2, that is, the biosolid treatment minus the one produced in the soil without biosolids (T0), which after 56 days was 1014.21 mg kg⁻¹ and 1654.73 mg kg⁻¹, respectively. At the end of the incubation, an average of 10.12% of the organic C of the biosolid was mineralized when added to the agricultural soil, while the treatment with only biosolid (T3) mineralized 4.67% of its organic C.

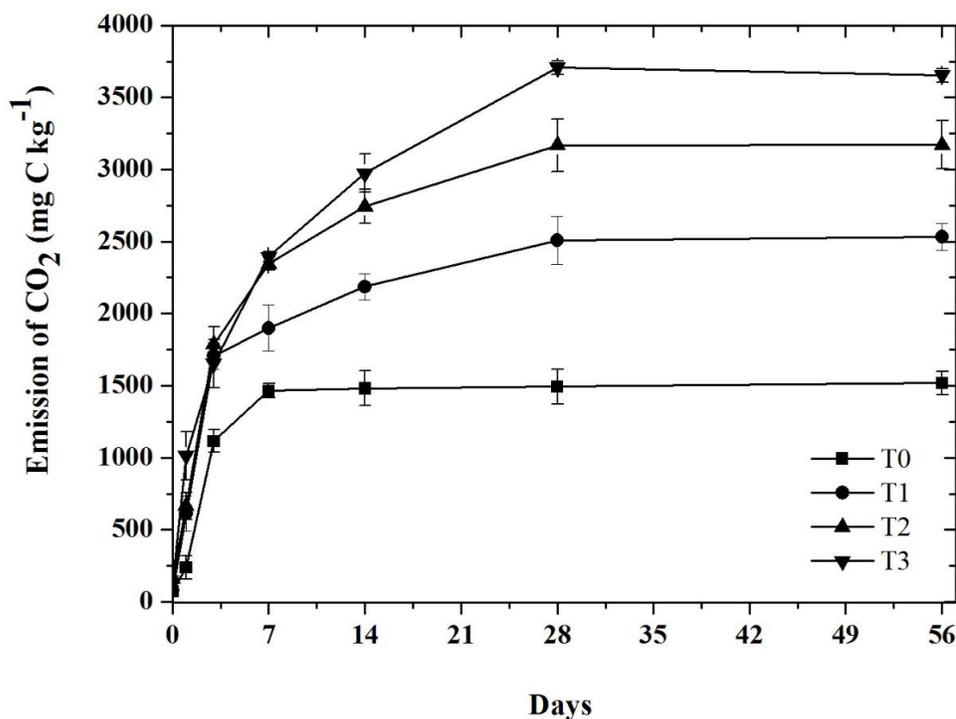


Figure 1. CO₂ emissions in treatments: control without amendment (T0), 100 mg NH₄⁺ kg⁻¹ (T1), 200 mg NH₄⁺ kg⁻¹ (T2), and biosolid (T3). The bars at each point represent the standard deviation (*n* = 3).
Source: Author's own elaboration.

The volatilization of NH₃ increased proportionally with the increase in the application rate of biosolids. The greatest volatilization occurred in the first 14 days of incubation (Figure 2). The total amount of NH₃ volatilized after 56 days was 12.75 mg kg⁻¹, 34.83 mg kg⁻¹, 38.85 mg kg⁻¹, and 64.68 mg kg⁻¹ for treatments T0, T1, T2, and T3, respectively. These values were 2.73 (T1), 3.05 (T2), and 5.07 (T3) times more than the volatilized NH₃ in T0. The T0 and T3 treatments showed significant differences (*p* ≤ 0.05) in the volatilization of NH₃, due to the fact that they are different matrices (soil and biosolid). Therefore, it was established that the volatilized NH₃ comes from the NH₄⁺ content of the biosolid added to the soil, with NH₃ volatilization percentages of 34.83%, 19.42%, and 7.52% for the T1, T2, and T3 treatments, respectively.

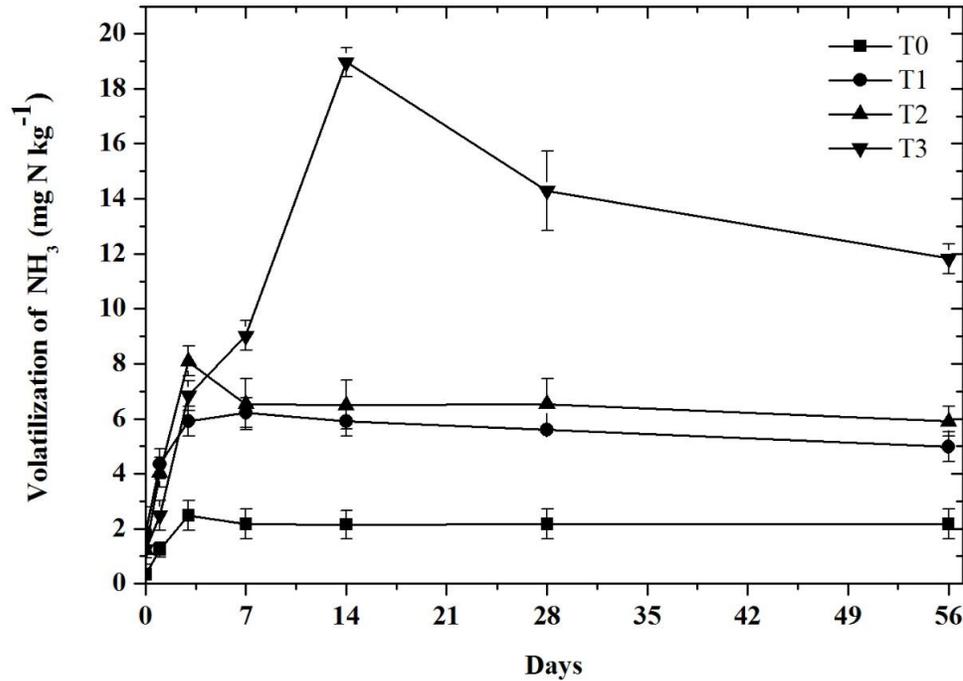


Figure 2. Volatilization of NH₃ in treatments: control without amendment (T0), 100 mg NH₄⁺ kg⁻¹ (T1), 200 mg NH₄⁺ kg⁻¹ (T2), and biosolid (T3). The bars at each point represent the standard deviation (*n* = 3).
Source: Author's own elaboration.

In each one of the biosolid treatments (T1, T2, and T3), the concentration of NH₄⁺ increased in the first days of incubation, then decreased, and increased again on day 56 (Figure 3a). The concentration of NH₄⁺ on dry soil from the control treatment (T0) remained < 30 mg kg⁻¹ during the incubation period. The average concentration of NH₄⁺ increased with the application rate of biosolid, although there was no significant difference (*p* ≤ 0.05) between T1 and T2 (75.84 mg kg⁻¹ and 84.05 mg kg⁻¹). However, there were significant differences (*p* ≤ 0.05) in the T0 and T3 treatments (20.36 mg kg⁻¹ and 163.85 mg kg⁻¹).

The concentration of NO₂⁻ in T0 remained < 3.5 mg kg⁻¹ from the beginning to the end of the incubation period. With respect to biosolids treatments (T1 and T2), the concentration of NO₂⁻ decreased in the first three days. On day 7, there were significant differences (*p* ≤ 0.05) in the concentration of NO₂⁻ for the T1, T2, and T3 treatments, with maximum concentrations of 9.83 mg kg⁻¹, 11.22 mg kg⁻¹, and 29.87 mg kg⁻¹, respectively. At the end of the incubation period, the concentrations of NO₂⁻ decreased to 3.04 mg kg⁻¹, 1.48 mg kg⁻¹, and 3.77 mg kg⁻¹ for the same treatments (Figure 3b).

The concentration of NO₃⁻ for the treatments remained constant (≤ 18 mg kg⁻¹) in the first days of incubation, except in T3, with a gradual increase from day 28 to reach concentrations of NO₃⁻ of 17.18 mg kg⁻¹, 48.28 mg kg⁻¹, 97.76 mg kg⁻¹, and 186.68 mg kg⁻¹ for T0, T1, T2, and T3, at the end of the incubation period. The average concentration of NO₃⁻ in the T1 and T2 treatments increased with higher added doses of biosolids (Figure 3c). The average amount of N available in the biosolid for the three treatments (T1, T2 and T3) was 3.27 g kg⁻¹, corresponding to 47.65% of its TN content.

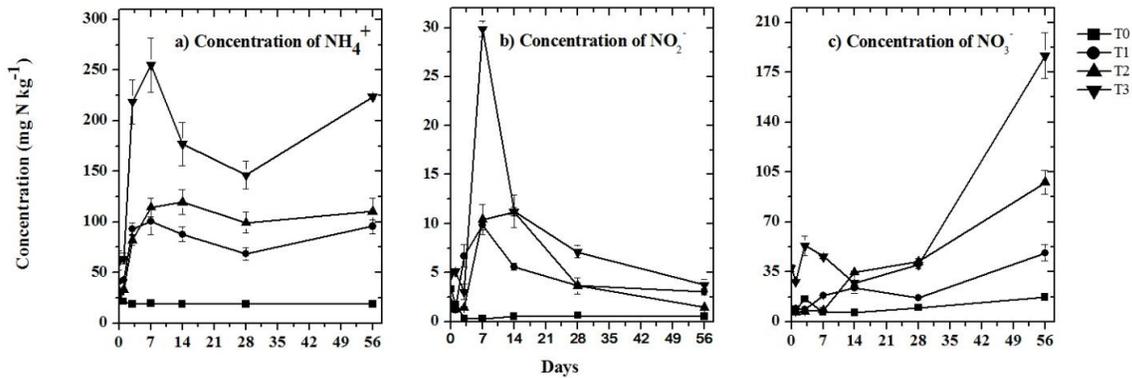


Figure 3. Concentration of inorganic nitrogen a) NH₄⁺, b) NO₂⁻, c) NO₃⁻ in the treatments: control without amendment (T0), 100 mg NH₄⁺ kg⁻¹ (T1), 200 mg NH₄⁺ kg⁻¹ (T2), and biosolid (T3). The bars at each point represent the standard deviation (*n* = 3).

Source: Author's own elaboration.

About MB-C, by day 7 of incubation, MB-C increased 8.7 and 9.4 times from its initial values in T1 and T2 treatments, while for T0 and T3 treatments it increased 3.6 and 3.2 times their value on day 14 of incubation, reaching maximum MB-C values of 500.47 μg g⁻¹, 1158.77 μg g⁻¹, 1429.65 μg g⁻¹, and 1587.4 μg g⁻¹ for T0, T1, T2, and T3, respectively (Figure 4a). No significant difference was found (*p* ≤ 0.05) between the T1, T2, and T3 treatments; however, the treatments were significantly different from the T0 treatment. The percentage of organic C of the biosolid added to the treatments incorporated in the MB-C was 6.57% and 3.95% for the T1 and T2 treatments, and 2.03% for the T3 treatment.

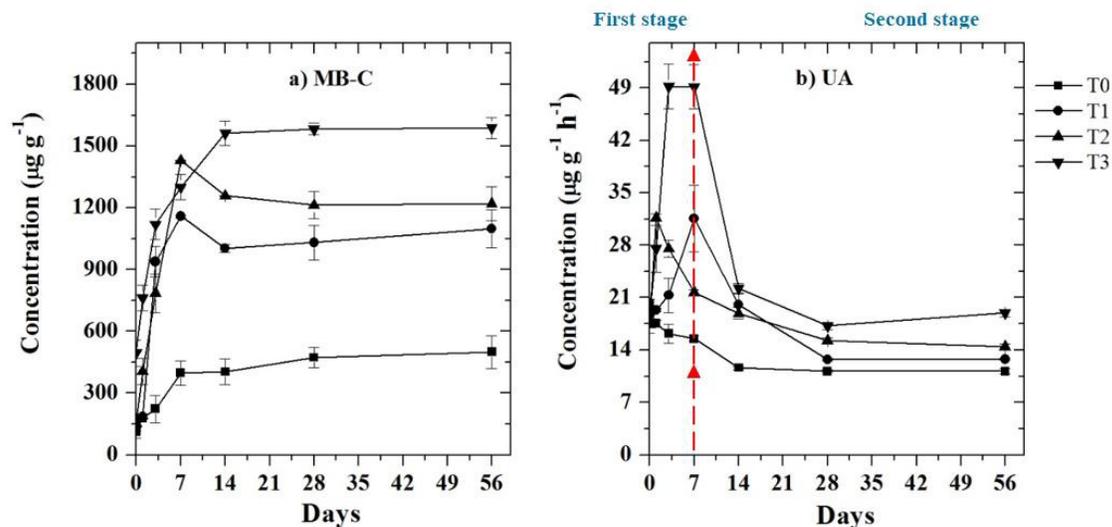


Figure 4. Biological properties a) Microbial biomass carbon (MB-C) and b) Urease activity (UA) in the different treatments: control without amendment (T0), 100 mg NH₄⁺ kg⁻¹ (T1), 200 mg NH₄⁺ kg⁻¹ (T2), and biosolid (T3). The bars at each point represent the standard deviation (*n* = 3).

Source: Author's own elaboration.

The average UA in the T0 and T3 treatments were significantly different ($p \leq 0.05$) from each other; nevertheless, they did not differ from the T1 and T2 treatments, so it can be assumed that the application rate of biosolids does not affect this parameter. However, UA increased in the first days of incubation for biosolid treatments (T1 on day 7 = 55.20%, T2 on day 1 = 81.40%, T3 on day 3 = 165.80%) compared to the non-biosolid treatment (T0) where this maximum activity occurred on day 0, and then gradually decreased (Figure 4b). With respect to the maximum values reached of UA based on the NH_4^+ content for T0, T1, T2, and T3 were $19.46 \mu\text{g g}^{-1} \text{h}^{-1}$ (day 0), $31.57 \mu\text{g g}^{-1} \text{h}^{-1}$ (day 7), $31.68 \mu\text{g g}^{-1} \text{h}^{-1}$ (day 1), and $49.21 \mu\text{g g}^{-1} \text{h}^{-1}$ (day 3), respectively. After reaching these values, the UA was reduced for all treatments by 45.50% on average with respect to the maximum values reached, the T0 treatment appeared as the one with the greatest decrease in UA.

Discussion

At the end of incubation, the pH values showed a slight decrease in soils amended with biosolid (T1 and T2), being higher according to the dose of biosolid applied. This may be due to the formation of organic acids as an effect of the addition of biosolid to the soil. A similar behavior was reported by Tamaríz *et al.* (2015), the pH of the unamended soil was neutral with a value of 7.1 and the soil amended with biosolids was slightly acidic with a value of 6.3. Roig *et al.* (2012) observed a decrease in pH as a result of the application of long-term biosolids to a carbonate-rich soil capable of buffering free acidity in the soil solution. This phenomenon was directly related to the mineralization of organic matter. A similar effect was seen on the soil of this study. On the other hand, it was found that the EC increased according to the dose of biosolid applied, which coincides with the results obtained in other studies (Rojas-Oropeza *et al.*, 2010; Sciubba *et al.*, 2014). However, these values would not imply salinity problems in the soil studied ($\text{EC} > 4 \text{ dS m}^{-1}$).

With reference to the dynamics of C, the average CO_2 emission did not differ significantly ($p \leq 0.05$) among treatments. As for the organic C of the biosolid applied to the soil, 10.12% was mineralized during the 56 days of incubation. Higher levels of organic C mineralization of biosolids were reported by Jin *et al.* (2011), who found mineralization levels of 11%–62% in their treatments after 120 days of application. Rojas-Oropeza *et al.* (2010) reported that 60% of the biosolids were mineralized in a saline-sodium soil of Lake Texcoco after 90 days of adjusting their CRA to 20% and an incubation temperature of 20 °C. Another study by López-Valdez *et al.* (2010) reported 16% and 27% biosolid mineralization for a saline-alkaline soil of Lake Texcoco with a pH of 10.3 and an agricultural soil of Otumba in the State of Mexico with a pH of 7.8, respectively. Differences in mineralization percentages may be due to differences in biosolids characteristics (TOC and TN), as well as soil properties both physical-chemical (pH, EC, and texture) and biological (rhizospheric activity, enzymatic activity, microbial biomass and its structure), or even to the incubation conditions of the experiment (incubation period, WHC, and incubation temperature) (Curtin *et al.*, 2014; Harrison-Kirk *et al.*, 2014).

With respect to the volatilization of NH_3 , the results obtained show an increase as the application rate of biosolid increases as well. However, at the beginning of incubation, the volatilization percentages of NH_3 derived from NH_4^+ of the added biosolids were found to be lower at higher application rates of biosolids. The average volatilized NH_3 value was 37.78%. Lower percentages of volatilization of NH_3 have been reported in other studies, reaching values of 8% to 17% of the total NH_4^+ present in the biosolids added in their experiments (Behera *et al.*, 2013; Guerra *et al.*, 2004). Within a period of 90 days of incubation, Rojas-Oropeza *et al.* (2010) reported values above 63% of NH_4^+ present in the biosolid added to the soil. The detriment of NH_3 by this mechanism may be due to different causes: 1) high soil pH, which increases the concentration of free NH_3 ; 2) environmental conditions such as soil aridity or moisture, high temperatures, soil texture; and 3) low soil cation exchange capacity (de Melo *et al.*, 2018; Rigby & Smith, 2014). In all treatments, the pH was maintained at values of 6.5 to 7.3, which are considered neutral, so the effect of pH for the release of free NH_3 is negligible. Therefore, the volatilization percentages of NH_3 observed in this study could be attributed to the intrinsic characteristics of the soil, such as texture or moisture, as reported by Peng *et al.* (2015).

In the same context, the concentration of NH_4^+ in soil is affected by different abiotic and biotic processes, for example, volatilization of NH_3 , fixation in soil matrix, immobilization of NH_4^+ , mineralization of N, and nitrification (López-Valdez *et al.*, 2010; Rigby & Smith, 2014). The concentration of NH_4^+ increased from the beginning of incubation to day 7 and 14, where it had a subsequent drop, which coincided with an increase in NO_3^- on those same days. This indicates that part of NH_4^+ was oxidized to NO_3^- (Heil *et al.*, 2016), which can be attributed to nitrification, where it was higher in T1 and T2 treatments than in T0 and T3. However, by day 56 the concentration of NH_4^+ increased again as did the concentration of NO_3^- . This may be due to the mineralization of a fraction of organic N that is difficult to assimilate within the biosolid, coinciding with the results obtained by Roig *et al.* (2012).

In the NO_3^- concentration dynamics, both the T0 treatment and the T1 and T2 biosolid treatments did not have an increase or decrease in the first 3 days of incubation. Such behavior could indicate that nitrification did not occur during this period. However, from day 7 onwards, the concentration of NO_3^- gradually increased until reaching concentrations on day 56 of 2.43, 6.01, 11.86, and 4.93 times the initial value for the T0 (17.18 mg kg^{-1}), T1 (48.29 mg kg^{-1}), T2 (97.76 mg kg^{-1}), and T3 (186.68 mg kg^{-1}) treatments. The processes of fixation and immobilization of N were not evaluated in this study, for which purpose the TN and nitrogen of microbial biomass during the dynamics would need to be determined (Pajares & Bohannan, 2016).

For MB-C, a significant difference was obtained between T0 and the rest of the treatments ($p \leq 0.05$), although an increase in this parameter was observed with respect to the application rate of the biosolid. This result coincides with the ones obtained by other authors and can be attributed to the stimulation of soil microbial activity due to the incorporation of nutrients and organic matter in the soil by the addition of biosolid (Celis *et al.*, 2013; Sciubba *et al.*, 2013; Xue *et al.*, 2015). The behavior of MB-C on day 7 for T1 and T2 could be attributed to the incorporation of exogenous microorganisms or easily assimilated substrates of the biosolid into the soil, stimulating of the natural activity of the soil microbiota and even a change in the structure of the microbial community (Azeem *et al.*, 2016; de Melo *et al.*, 2018). It may also be due to a phenomenon known as the "priming effect" (Celis *et al.*, 2011; Rojas-Oropeza *et al.*, 2010). The phenomenon of a stimulation of the mineralization of organic matter that is incorporated into the soil was called the "priming effect" by Bingeman *et al.* (1953). This means that the priming effect was reflected in the increase in decomposition rate of soil organic matter after adding the biosolids to the soil, linked to an increase in microbial biomass as a consequence of greater availability of energy released by the decomposition of organic matter in the biosolids added. Thus, an increase in both CO_2 emission and C-MB from day 7 can be observed in Figure 1 and Figure 4a, respectively.

With reference to UA, an increase was observed for biosolid treatments (T1, T2, and T3) in the first seven days of incubation, followed by an average decrease of 45.50% by the end of the period. These results coincide with those obtained by Adetunji *et al.* (2017), which showed that UA increases with nitrogen-rich organic fertilization such as biosolids (C/N = 11.36) and decreases with soil tillage. In addition, the higher UA value was not affected by the biosolid dose applied. However, at higher doses (T2) this value was reached by day 1, whereas for treatment with lower doses (T1) it was reached until day 7. This may be associated with the total organic nitrogen content present in the treatments (T0 = 1956.3 mg kg⁻¹, T1 = 2642.0 mg kg⁻¹, T2 = 3327.7 mg kg⁻¹, and T3 = 5891.0 mg kg⁻¹). This statement coincides with the results obtained by Celis *et al.* (2011), where they associated high levels of urease with a high content of nitrogen compounds in the wastewater sludge evaluated. The behavior of UA in this study can be divided into two stages. In the first stage it increased 100% on average for the T1, T2, and T3 treatments in the first 7 days, as a result of the rapid mineralization of the nitrogen content in the biosolids. In the second stage, an average reduction of 45.50% was present in all treatments until the end of incubation. This could be attributed to a decrease in the synthesis of this enzyme, due to the increased concentration of NH₄⁺ (Chen *et al.*, 2018; Núñez *et al.*, 2012; Redmile-Gordon *et al.*, 2015). It may also be due to rapid mineralization of easily assimilated N. The inhibition by NH₄⁺ of the synthesis of urease and other N-metabolizing systems in various microorganisms are linked to a complex bicyclic cascade controlled by the glutamine formed by the glutamine synthetase activity associated with NH₄⁺ assimilation (Chuckran *et al.*, 2021). McCarty *et al.* (1992) evaluated the effects of different amounts of inorganic N on urease production in soils amended with glucose, and the author showed that, although microbial metabolism measured by CO₂ production was stimulated by the addition of different doses of NH₄⁺ to amended soils, urease production was decreased at a higher dose of N. However, Diacono & Montemurro (2011) mentioned that inorganic N forms (NH₄⁺ y NO₃⁻) are released over a longer period (> 90 days), which does not coincide with the results obtained in this study for NH₄⁺. This was produced from the start of the dynamics until reaching its highest values for all treatments between day 7 and 14 (Figure 3), coinciding with the decrease in urease activity (Figure 4b).

Conclusions

The application of biosolids to agricultural soil led to a stimulation of soil microbial activity. The CO₂ emissions and MB-C increased after applying the biosolids to the soil, and the increase was proportional to the application rate. Moreover, the addition of biosolids to the soils studied did not affect the pH. On the other hand, the EC was affected and, although the values reached do not represent salinity problems, it is recommended to monitor the EC in soils amended with biosolids in this region. The application of biosolids in the soil improved the quality of C and N content available, which allows for better crop growth. In addition, it was observed that the mineralization of biosolids has two forms of mineralizable organic nitrogen, so the supply of nutrients is favored over time. Easily assimilated nitrogen content stimulates an increase in UA due to the rapid mineralization of this, however, it can also be inhibited over time by the increase of inorganic nitrogen in the soil. Finally, to better establish the existence of a positive or negative effect of the addition of biosolids on the microbial activity of soils, it is necessary to conduct additional studies of both the chemical composition of the biosolids and their mineralization in order to determine such effects and thus establish good practices for their use.

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Conflicts of interest

The authors have declared that no competing interests exist.

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