

Microbiological activity and N transformations in a soil subjected to aggregate extraction amended with pig slurry

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Pig slurry as a fertilizer source has been extensively used in agriculture; however, in order to reduce the risks of contaminating the water supplies given its high level of N sources, it is important to understand the N transformations occurring in the soil where it is applied. In this study, incubations were performed at 25 °C for a period of 63 to 73 d to evaluate the effect of different doses of pig slurry on the global microbiological activity and the N dynamics in time, with an emphasis on N mineralization and nitrification in a soil subject to aggregate extraction. The slurry was applied in doses equivalent to: 0, 162, 244, and 325 m³ ha⁻¹, constituting four treatments: T0, T50, T75, and T100, respectively. The microbiological activity and the contents of NH₄⁺-N and NO₃⁻-N were measured. Increasing doses of slurry produced an increase in the evolution of the accumulated CO₂, with 63.5, 115.0, 112.7, and 125.7 mg 100 g⁻¹ soil for T0, T50, T75, and T100 respectively. A similar situation was observed in the initial contents of NH₄⁺-N, which were 22.4, 30.3, 44.3, and 60.7 mg kg⁻¹ in each treatment, respectively. On the other hand, the increase in NO₃⁻-N contents were only noticed by the end of the incubation period and corresponded to 28.6, 69.0, 95.3, and 109.8 mg kg⁻¹. In addition, the net N mineralization was predominant in all treatments with slurry during the measurement period, being 9.1, 45.4, 58.1, and 52.7 mg kg⁻¹ for T0, T50, T75 and T100, respectively, at the end of the trial. The mineralization rate of the organic C decreased when increasing the dose of slurry and the mineralization rate of the organic N resulted to be low, which would indicate a high contribution of material resistant to degradation by the slurry, which could have a long term effect in the soil.

Key words: Degraded soil, microbial activity, N mineralization, N immobilization, pig slurry.

INTRODUCTION

Pig production in Chile has developed mainly due to their domestic consumption and, from several years ago to their export, where it has been sought to reach the high standards of the international markets (INE, 2013; ODEPA, 2013). This situation has caused a sustained increase in the volumes of pig slurry, the main residue of pig farms, and its disposal has turned into a health and environmental problem.

Pig slurry, which corresponds to a mixture of excreta, food debris, rinse water and beds and soil of the animals (Peralta, 2005) presents a variable composition, depending on the type of production system, climate, and size, age and breed of the animals, in addition to the type and quantity of feeding. However, some of the characteristics common to this waste are its high water and high ammonium contents (Sánchez and González, 2005).

In Chile there are no specific rules to treat the slurry, but if companies conduct a misuse, they are likely to violate some rules of the Chilean legislation affecting agriculture, health and public works, among others. There are several technologies for the treatment of slurries that vary in complexity, cost, and number of operations (Varnero et al., 2009). However, there is currently no fully consolidated technology that would serve as a reference for dealing with these wastes. For that reason, there are certain policies for improving the porcine sector, which have been translated into the design of a Clean Production Agreement (CPA) that includes an Implementation Plan for Slurries, as a way of facilitating pig farms to achieve international environmental standards by improving the levels of competitiveness in the sector, community health and quality of the national environment (CNBPA, 2003).

The application of pig slurry to soils is one of the most commonly used practices in agriculture, due to its low cost and fertilizer value, which is based on its high content of ammonium forms, which are transformed by the microorganisms present in the soil into nitrates, the main way of N absorption by the roots of plants (Plaza et al., 2005). However, the lack of planning in the application of slurries to soils promotes the contamination with pathogens (Amin et al., 2013), bad odors, proliferation of insects and eutrophication of waters (Varnero et al., 2009),

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in addition to groundwater contamination by leaching of nitrates, pathogens (Mantovi et al., 2006; Amin et al., 2013) and an uncontrolled increase in greenhouse gases emissions (Bertora et al., 2008).

Soil subjected to aggregate extraction could be a good alternative for application of pig slurry. The slurry could be utilized as N source in establishing crops on these soils, as part of a rehabilitation program. In the last decades these types of soils have increased, since there has been a growth in aggregate extraction tasks, especially in the Metropolitan Region, Chile, as a result of the expansion of the city (Martínez, 2003).

During aggregate extraction from agricultural soils, cutting of vegetation and soil movement is produced, causing a loss of vegetation cover and soils; as a result, the soil surface loses its structure, can change its texture and might decrease its level with respect to the original soil, thus reducing the distance to the water table (Adasme, 2002). This is why in these types of soil, a boost is necessary for their recovery, which is based on the use of organic amendments that would contribute to improve their physical, chemical, and biological properties (Gálvez et al., 2011). In this context, pig slurries arise as an alternative to N fertilization of easy implementation and low cost, which could be considered in fertilization plans (Lopez-Ridaura et al., 2009).

Implementation Plan for Slurries establishes a characterization of the residue, an identification of the generation source prior to soil application (CPL, 2005). For these reasons it is convenient to know the effects of applying different doses of pig slurry on the global microbiological activity and transformations of soil N. This type of study could contribute to reduce the excess of N with the subsequent leaching of nitrates and groundwater contamination in the soils resulting from aggregate extraction. For this purpose, incubations in the laboratory of soil mixed with pig slurry, under controlled conditions of moisture and temperature, have been shown to be useful tools delivering valuable information on the potential C and N mineralization (Busby et al., 2007).

Therefore, the objective of this work was to study how different doses of pig slurry affect the global microbiological activity and the N dynamics in time, with an emphasis on N mineralization and nitrification in a soil subjected to aggregate extraction in aerobic laboratory conditions.

MATERIALS AND METHODS

The soil used in this experiment was obtained from the 30 cm surface layer of an anthropic soil. This soil is classified as Typic Xerochrepts, Inceptisol (Soil Survey Saff, 2003) generated by aggregate extraction in Germán Greve Silva Experimental Agricultural Station, located in Rinconada de Maipú (33°28' S, 70°50' W). A general characterization of a composite soil sample was performed, as shown in

Table 1 (CNA, 2004). The soil was air-dried, sieved to 2 mm and kept at 5 °C.

The slurry was collected from a pig farm with a confined-animals production system located on the road to Lonquén. A general characterization of a composite sample of pig slurry was performed (APHA, 2005) (Table 1). To determine the effect slurry, the soil was mixed manually with different doses of slurry, these were compared with a control treatment without addition of slurry, establishing four treatments. The doses used corresponded to three dilutions of slurry in water: 50%, 75%, and 100% of slurry. T0: Soil + 0% slurry (control treatment), T50: Soil + 50% slurry (equivalent to 162 m³ slurry ha⁻¹), T75: Soil + 75% slurry (equivalent to 244 m³ slurry ha⁻¹) and T100: Soil + 100% slurry (equivalent to 325 m³ slurry ha⁻¹).

Determination of global microbiological activity and organic C of soil and pig slurry mixtures

The global microbiological activity in the soil was determined as CO₂ evolution (CO₂-C 100 g⁻¹ soil) by the method proposed by Anderson (1982), whose principle is based on the capture of CO₂ produced by respiration from a wet soil in a 0.1 M NaOH solution. The amount of NaOH remaining after a known incubation period is determined by titration with a standard acid. This procedure was performed with 25 g soil samples with a water content adjusted to 80% of the available moisture, put into plastic containers each 1L in triplicate, for aerobic incubation experiment at 25 °C, for a period of 73 d, changing the alkaline solution every 2 or 3 d; from day 15, determinations were performed every 5 to 7 d. The percentage of organic C was determined by wet combustion, at the beginning and at the end of the incubation period. This method is based on the oxidation of the organic matter of the sample using sodium dichromate in a strong sulfuric acid medium. After the occurrence of the reaction, the reduced chromate is colorimetrically measured (CNA, 2004). The mineralization rate of the organic C at the end of the incubation period was calculated as the ratio between the percentage of C evolved as accumulated CO₂ in each treatment at the 73 d of incubation and the percentage of organic C at the beginning of the treatments.

Table 1. General characterization of pig slurry and soil subjected to aggregate extraction.

Analysis	Soil	Pig slurry
Textural class	Sandy loam	-
pH water (soil 1:2.5)	8.0	6.7
Organic matter, % (Walkley-Black method)	1.05	-
Organic matter, % (calcination method)	2.82	47.0
Organic C, g L ⁻¹	-	27.8
Electrical conductivity, dS m ⁻¹	0.3	21.5
Density, Mg m ⁻³	1.29	1.03
Total N, %	0.06	0.25
Total N, g L ⁻¹	-	2.50
NH ₄ ⁺ -N, mg kg ⁻¹	0.2	1.182
NO ₃ ⁻ -N, mg kg ⁻¹	22.2	24.0
C/N	10.2	11.1
Total solids, %	92.5	7.7
Soluble solids, %	-	5.1

Determination of N mineralization of soil and pig slurry mixtures

The mineral N levels (NH_4^+ and NO_3^-) (mg kg^{-1} soil) were measured in 25 g soil samples with a water content adjusted to 80% of the available moisture, put into polyethylene bags in triplicate, for aerobic incubation experiment at 25 °C; the sampling was performed every 7 d over a period of 63 d for each treatment according to Bremner (1996). This method is based on an extraction step with 2 N KCl; a posterior steam distillation with magnesium oxide (MgO) for determining NH_4^+ and an addition of Devarda's alloy in the same extract for another steam distillation. Finally, the extract was titrated with a standardized acid for both determinations. Inorganic N was calculated adding $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$. The organic N was obtained from the difference between the total N and the mineral N for each treatment. Additionally, net N mineralization was obtained by the difference between the mineral N at the end and at the beginning of the incubation period for each treatment. The mineralization rate of the organic N at the end of the incubation period was calculated as the ratio between the percentage of net mineralization at the end of the incubation period and the percentage of organic N at the beginning of each treatment.

Experimental design and data analysis

A completely randomized design consisting of four treatments (control, 50%, 75%, and 100% of slurry) with three replicates each was conducted. The experimental unit used to determine the global microbiological activity corresponded to a composite soil sample placed inside a plastic container. In the case of mineral N determination, a composite soil sample placed in a polyethylene bag was used.

After checking the normality and homogeneity assumptions of the variances, data were analyzed using ANOVA ($P \leq 0.05$) and Tukey's multiple comparison test ($P \leq 0.05$) when significant differences were observed between treatments. The statistical analysis was done using the software Statgraphics Plus (StatPoint Technologies, Warrenton, Virginia).

RESULTS AND DISCUSSION

Global microbiological activity: mineralized $\text{CO}_2\text{-C}$

The addition of pig slurry to the soils caused an increase in the evolution of CO_2 compared to the control treatment (T0). The major CO_2 evolutions occurred during the first 43 days, after a short lag phase in the soils treated with slurry and a most extensive one in the control treatment (Figure 1), which is consistent with the contribution of easily degrading C and N sources by the slurry (Amon et al., 2006), in addition of exogenous heterotrophic microorganisms present in the waste. On the other hand, the rapid increase of CO_2 in the early days, would be also due to the wetting and sieving of the soil, which generates aeration and exposes organic C fractions which in the soil

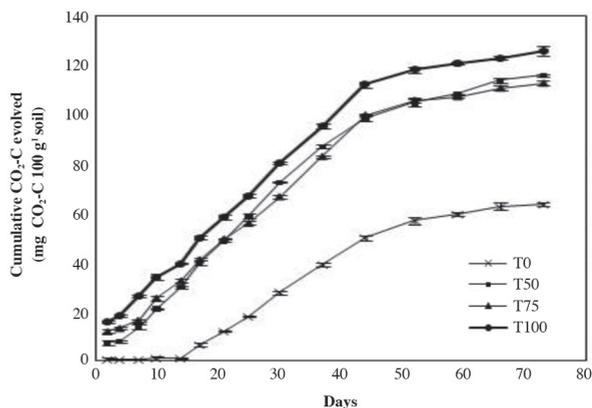


Figure 1. Evolution of accumulated $\text{CO}_2\text{-C}$ during the incubation period of 73 d under controlled laboratory conditions in a soil subjected to aggregate extraction with different doses of pig slurry. Bars correspond to the standard deviation ($n = 3$).

would be protected from the action of microorganisms, similar results were found by Cayuela et al. (2009). From day 44 the evolution of CO_2 was reduced, which is related to the depletion of labile C and N sources and, moreover, with the prevalence of microbial activity related to the sources of C and N more resistant to microbial degradation (Guerrero et al., 2007).

The highest accumulated respiration was found in the treatment with the highest dose of slurry (T100), which would contain the greatest amount of easily degradable organic compounds supplied by the slurry (Halil et al., 2015). No significant differences were observed between treatments T50 and T75 (Table 2), which was also observed during the incubation period (Figure 1).

The mineralization rates of organic C for T75 and T100 resulted significantly lower than the control, which could be attributed to the existence of C sources more resistant to microbial degradation, supplied by the slurry; while T50 presented the highest mineralization rate of organic C (Table 2).

Regarding the organic C at the beginning of the incubation period, the highest value was determined in treatments T75 and T100 (Table 3), which contained the highest amounts of organic C provided by the slurry, without significant differences between them, although T100 organic C > T75 organic C. A similar situation was observed in treatments T50 and T75, T75 organic C > T50 organic C. The control treatment presented an organic C

Table 2. Cumulative $\text{CO}_2\text{-C}$ mineralization and mineralization rate of organic C at the end of the incubation period of 73 d in an anthropic soil subjected to aggregate extraction.

Treatments	$\text{CO}_2\text{-C}$ mineralization ($\text{mg } 100 \text{ g}^{-1}$ dry soil)	Mineralization rate of organic C
T0: Soil + 0% Pig slurry	63.5c	110.41b
T50: Soil + 50% Pig slurry	115.0b	13.58a
T75: Soil + 75% Pig slurry	112.7b	9.38bc
T100: Soil + 100% Pig slurry	125.7a	8.27c

Different vertical lower-case letters indicate significant differences ($P \leq 0.05$) among the different treatments for the mineralized $\text{CO}_2\text{-C}$ and the mineralization rate of organic C, respectively.

Table 3. Organic C at the beginning and at the end of the incubation period of 73 d in an anthropic soil subjected to aggregate extraction.

Treatments	Organic C (%)	
	Day 0	Day 73
T0: Soil + 0% Pig slurry	0.61Ac	0.60Aa
T50: Soil + 50% Pig slurry	0.86Abc	0.65Ba
T75: Soil + 75% Pig slurry	1.20Aab	0.83Aa
T100: Soil + 100% Pig slurry	1.52Aa	0.81Ba

Different vertical lower-case letters indicate significant differences ($P \leq 0.05$) among different treatments for organic C. Different horizontal capital letters indicate significant differences ($P \leq 0.05$) for organic C between the beginning and the end of the incubation period for an equal treatment.

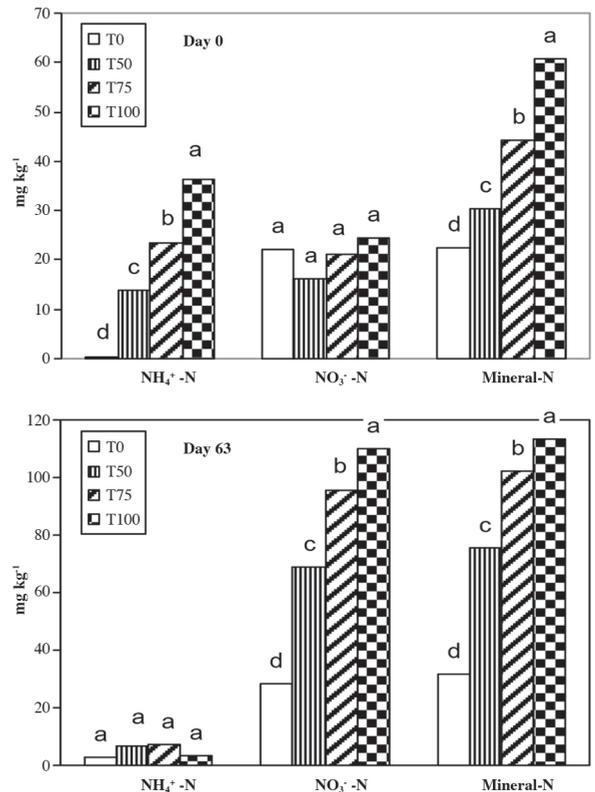
content significantly lower compared with T75 and T100. However, it was observed that increasing amounts of slurry tended to increase the organic C.

For the same treatment, at the beginning and at the end of the incubation period, a decrease in organic C was observed in all treatments with slurry, being significant for T50 and T100 (Table 3). Thus, in the slurry treatments, the decrease percentages of the organic C with respect of the initial organic C were 24.4%, 30.8%, and 46.7% for treatments T50, T75, and T100, respectively. This decrease in organic C would be a consequence of a loss of C measured as CO_2 evolution from the labile organic C provided by the pig slurry and also by the C assimilated by the microbial biomass (Plaza et al., 2007). In the control treatment no reduction in the organic C was observed, which was expected since there were no labile C entries to the system and because the measure of organic C from soils is poorly sensitive to changes over short periods of time (Haynes, 2005).

Dynamics of mineral N

The concentrations of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ of the control and the slurry-treated soils at the beginning and at the end of the trial are shown in Figure 2. It was observed that the initial addition of pig slurry significantly increased the content of $\text{NH}_4^+\text{-N}$, especially in those treatments with higher doses. Similar results were found by Plaza et al. (2005), Guerrero et al. (2007) and Fangueiro et al. (2014). Plaza et al. (2005) and Fangueiro et al. (2014), when adding the pig slurry to the soil and Guerrero et al. (2007) when using the solid fraction of the slurry. This situation was not observed in the $\text{NO}_3^-\text{-N}$ content, due to the small amount of it provided by the slurry. Thus, the $\text{NH}_4^+\text{-N}$ constituted from a 0.89% in the control treatment to a 59.8% in T100. Conversely, at the end of the incubation period, the $\text{NO}_3^-\text{-N}$ content was the one that increased significantly in the treatments with higher applications. In this case, the $\text{NO}_3^-\text{-N}$ constituted from 90.8% in the control treatment to 96.8% in T100.

In synthesis, nitrification occurred in all treatments and was significantly higher in those with slurry applications, given the high content of $\text{NH}_4^+\text{-N}$ provided by this waste and by the mineralizable organic N in the short term. For that reason, a decrease in the $\text{NH}_4^+\text{-N}$ content was observed in time (Figure 3), followed by an increase of



Different lower-case letters indicate significant differences ($P \leq 0.05$) among the different treatments for $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and mineral N for days 0 and 63, respectively.

Figure 2. $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and mineral N at the beginning and at the end of the incubation period of 63 d for the different doses of pig slurry in an anthropic soil subjected to aggregate extraction.

$\text{NO}_3^-\text{-N}$ (Figure 4), whose content would be also related to the action of nitrifying microorganisms from the beginning of the incubation period.

Regarding the mineral N, an initial increase was observed after the application of the pig slurry, which is related to the contribution of $\text{NH}_4^+\text{-N}$ by these residues; later, during the incubation period, fluctuations occurred

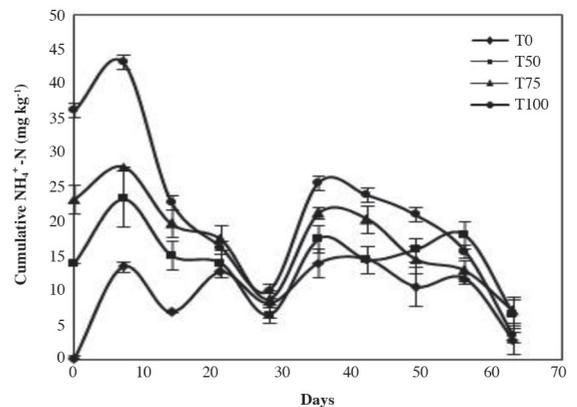


Figure 3. $\text{NH}_4^+\text{-N}$ evolution in samples incubated for a period of 63 d for treatments T0, T50, T75, and T100. Bars indicate standard deviation ($n = 3$).

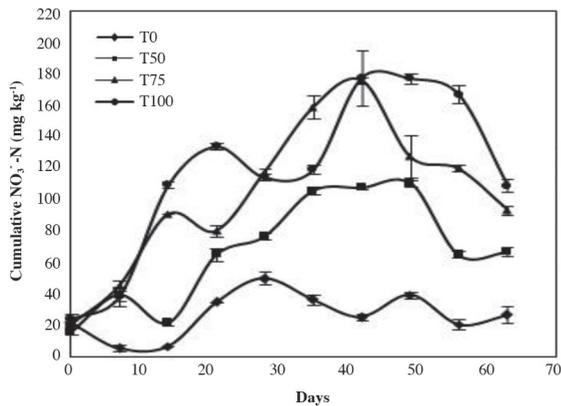


Figure 4. $\text{NO}_3\text{-N}$ evolution in samples incubated for a period of 63 d for treatments T0, T50, T75, and T100. Bars indicate standard deviation ($n = 3$).

which are attributed to a transient immobilization by the microbial biomass. Finally, the mineral N increased due to an increase in the concentration of $\text{NO}_3\text{-N}$ and, because of that, during the incubation period the mineral N was determined by the dynamics observed for the $\text{NO}_3\text{-N}$ (Figure 5).

Net mineralization and net N mineralization rate

The highest values of net N mineralization were obtained in T75 and T100 with no significant differences between these treatments. On the other hand, the lowest value was obtained in the control treatment, with significant differences between the samples treated with slurry compared to the control (Figure 2).

In this study, no significant differences in the mineralization rate of the organic N between treatments were observed (Figure 2); although there seemed to be a tendency of a lower mineralization rate when increasing the doses of pig slurry, similar to the results found by Plaza et al. (2005). This would indicate that a high proportion of organic N in the pig slurry would be

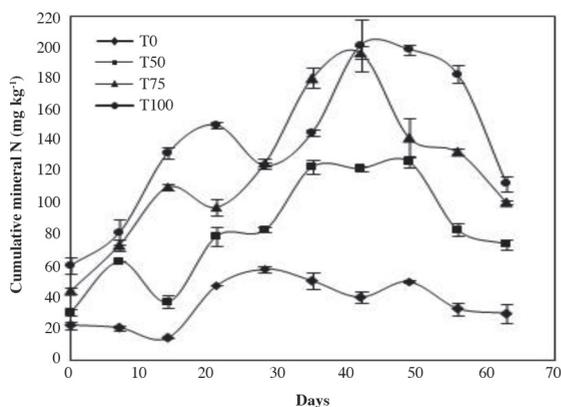


Figure 5. Mineral N evolution in samples incubated for a period of 63 d for treatments T0, T50, T75, and T100. Bars indicate standard deviation ($n = 3$).

resistant to mineralization and, hence, this fraction may have a long-term effect on the soil.

On the other hand, the results of the mineralization rate of the organic N are consistent with those obtained in the mineralization rate of the organic C, where a higher dose of pig slurry resulted in a lower mineralization rate. Furthermore, it has been reported that increasing concentrations of mineral N could inhibit the degradation of recalcitrant materials (Carreiro et al., 2000), which could explain the mineralization rate of the organic C and mineralization rate of the organic N results in this study.

In the present study, net immobilization only occurred in the control treatment (T0) on the first 2 wk, with net mineralization being observed at all sampling times in the treatments with pig slurry (Figure 6), being the net mineralization higher in the soils treated with slurries respect to the control. These results differ from those obtained in other studies (Plaza et al., 2005; Fangueiro et al., 2010), where net mineralization was higher and in some cases equal in the control soils with respect to the pig slurry amended soils, which showed N immobilization at various sampling times.

The absence of an initial net immobilization in the slurry treatments could be explained by the addition of easily degradable C sources and by the microbial population supplied by the pig slurry (Amon et al., 2006), suggesting that this could be related to the increased net mineralization observed in these treatments. While it is true that in the treatments amended with pig slurry no net immobilization was determined, this does not mean that this process has not occurred, but rather that mineralization was quantitatively greater, since both processes occur simultaneously (Amlinger et al., 2003; Haynes, 2005).

In general it is accepted that C/N of the waste, together with their type and biochemical composition, is related to the amount of N released, where ranges of C/N are set to define the process of mineralization-net N immobilization. Thus, C/N values $\leq 20\text{-}30$ would promote

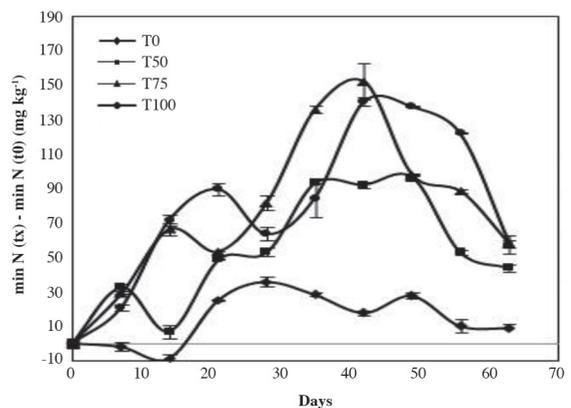


Figure 6. N mineralization evaluation for treatments: T0, T50, T75, and T100. Bars indicate standard deviation ($n = 3$).

N mineralization; however, C/N values ≥ 30 -40 would favor N immobilization processes. Hence, in the pig slurry with a C/N of 11.1, net mineralization predominates, increasing when rising the amount of slurry applied to the soil (Murphy et al., 2011).

Furthermore, a significant correlation between accumulated CO_2 -C and mineral N between treatments ($r = 0.88$) and between accumulated CO_2 -C and net N mineralization ($r = 0.96$) was observed. In general, a positive correlation was expected between these variables, as they are closely related to the metabolic activity that would come from the active component of the organic matter, and would reflect the accumulation of labile organic C and N and NH_4^+ resulting from the addition of pig slurry to the soil.

CONCLUSIONS

The application of crescent doses of pig slurry to the soil significantly increased the initial pool of organic carbon, by raising the microbial activity measured as CO_2 evolution. A similar increase occurs with ammonia, which is rapidly oxidized to nitrate, producing a parallel increase of mineral N in the short term; this situation is of great importance due to the high mobility of NO_3^- -N in the soil. Additionally, the rate of organic N mineralization was low, indicating that the slurry may contain levels of slowly-degrading organic N for microorganisms, which could have long term effects on the soil.

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