

Contribution of nitrogen mineralization indices, labile organic matter and soil properties in predicting nitrogen mineralization

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Abbreviations: N, nitrogen; N_0 , potentially mineralizable nitrogen; N_{0kf} , potentially mineralizable nitrogen determined by a fixed constant rate; k , constant rate; SOM, soil organic matter; POM, particulate organic matter; SOC, soil organic carbon; SON, soil organic nitrogen; C:N, carbon-nitrogen ratio of soil; POM-C, total particulate organic carbon; POM-N, total particulate organic nitrogen; cPOM-C, coarse particulate organic carbon; cPOM-N, coarse particulate organic nitrogen; fPOM-C, fine particulate organic carbon; fPOM-N, fine particulate organic nitrogen; CH, soil carbohydrates; CHt, total carbohydrates; CHs, soluble carbohydrates; gNmin, net N mineralization; Nan, anaerobic nitrogen; Nhyd, hydrolyzable N; NO_3^- -N, cold nitrate; N_{205} , N determined by spectrometer at 205 nm; N_{260} , N determined by spectrometer at 260 nm; Pe, extractable P; BD, soil bulk density; NT, no-tillage system.

Abstract

The objectives of this study were i) to measure the nitrogen (N) mineralization potential; ii) to evaluate N mineralization indices and labile soil organic matter (SOM) fractions, and iii) to analyze their interrelationship with soil properties for predicting the potential N mineralization in Mollisols under no-tillage of the Argentine Pampas. Potential N mineralization pool was determined by aerobic incubation of soil samples from 0-20 cm depth at 25°C for six drying and rewetting cycles over 21-24 weeks. The data were fitted to a first-order exponential equation to determine the potentially mineralizable N at a variable (N_0) and at a fixed constant rate (N_{0kf}). Several N mineralization indices, labile organic fractions and soil properties were determined. A broad range of values were found for N_0 (29.9-220.7 mg kg⁻¹) and N_{0kf} (26.9-172.4 mg kg⁻¹). A close relationship ($P < 0.001$) was found between N_0 and N_{0kf} with a high coefficient of determination ($R^2 = 0.94$). High R^2 values were found for predicting N_0 and N_{0kf} using the principal component as regress variable. Total and labile organic fractions and sand content, explained the majority of the variance. No single N mineralization index, labile organic fraction or soil property can accurately predict the potential N mineralization at a variable and at a fixed constant rate. The potential N mineralization pool may be controlled mainly by the variables related to the size of SOM and some labile fractions, *i.e.* soil organic C (SOC) and N (SON), soluble carbohydrates (CHs) and fine particulate organic N (fPOM-N) in these Mollisols.

Keywords: Potentially mineralizable N, Soil organic matter, Principal component analysis, Mollisols

1. Introduction

The Argentinean Pampas region is regarded as one of the most important grain-producing areas worldwide, with wheat (*Triticum aestivum* L.), maize (*Zea mays* L.) and soybean (*Glycine max* L. Merr.) being the main crops (Satorre and Slafer, 1999). Enhancing nitrogen (N)-use efficiency is also essential, particularly in semiarid and semihumid areas where fertilizers are rather costly and their efficiency is limited by low water availability. To determine rates of N fertilizer it is necessary to take into account the inorganic N in the soil and the organic N mineralized during the crop growing season (Martínez and Galantini, 2017). In particular, an accurate estimate of the N mineralized from soil organic matter (SOM) will allow to determine the rate of N fertilizer to optimize crop yield.

Most estimates of N supply through mineralization are based on aerobic incubation under controlled conditions over a long period of time (Stanford and Smith, 1972; Martínez and Galantini, 2017). This method is used to determine the soil N fraction capable of being transformed into mineral forms, also referred to as potentially mineralizable N (N_0). This is the labile pool of organic N considered to be a standardized estimate of potential mineralization in soil (Ros *et al.*, 2011; St. Luce *et al.*, 2011), *i.e.* it reflects the amount of organic N that can be mineralized.

A number of chemical and biological methods have long been applied to identify the potential mineralization of soil organic N in a quick simple way (Martínez and Galantini, 2017). Moreover, laboratories refuse to apply these methods on the grounds that they are rather time-consuming; modern soil testing laboratories, instead, strive for a short turn-around time and faster procedures. Biological indices such as anaerobic incubation are sensitive to different

soil managements (Moya *et al.*, 2016; Martínez *et al.*, 2017). Chemical methods are based on the hypothesis that a specific extractant releases N from a pool, but they do not usually require that the N pool released by extraction should be chemically homogeneous or biologically significant (St. Luce *et al.*, 2011). Some authors (Gianello and Bremner, 1986) have proposed that soil digestion with a strong salt can be used to estimate soil N-mineralization. But chemical methods cannot simulate the action of microorganisms and they do not account for the effects of environmental conditions (Sharifi *et al.*, 2007).

Soil organic matter and its labile fractions are considered important factors in regulating N-dynamics (Ramírez *et al.*, 2016; Martínez *et al.*, 2017) in view of their key role in N mineralization and N availability to crops. Available soil N is primarily produced by mineralization of SOM (Martínez-Lagos *et al.*, 2015), which supplies 50 to 80% of the N needed by the crop (St. Luce *et al.*, 2011). Soil organic N (SON) accounts for as much as 95% of total N in the topsoil. This organic fraction enters the soil as particulate organic matter (POM) through above-ground plant litter and root litter (Martínez *et al.*, 2017). The POM may provide more accurate information on N mineralization as it contains easily mineralizable N fractions. This fraction represents the mineralizable pool and it can easily predict the N mineralization capacity (Martínez *et al.*, 2017). Haynes (2005) reported that POM is an important labile N pool in several soils. However, decomposition of this labile fraction is heavily dependent on residue input and weather conditions (Martínez *et al.*, 2017), which vary significantly between years in semiarid and semihumid areas.

None of the mineralization indices or SOM fractions has been universally accepted as an indicator

of the soil's capacity to supply N and substantial uncertainty exists on their role in the N-cycle (Ros *et al.*, 2011). On the other hand, chemical and physical properties could affect the N mineralization (St. Luce *et al.*, 2011). However, understanding their function and interrelationships with other soil properties in the N-cycle may improve our ability to predict the N mineralization. For this reason, combining these properties in multiple regression (MR) models, could improve the prediction of N mineralization in different soils (Schomberg *et al.*, 2009; Ros *et al.*, 2011). An extensive number of studies have related N mineralization to soil properties and climate conditions (Schomberg *et al.*, 2009). Combining SOM fractions and soil properties into MR models has been shown to improve the prediction of potential N mineralization (Schomberg *et al.*, 2009; Dessureault-Rompré *et al.*, 2010; Nyiraneza *et al.*, 2011). However, as was mentioned by different authors (Shukla *et al.*, 2004; Ros *et al.*, 2011) using MR could result in multicollinearity between the factors of the model, confusing the biological sense of each variable in the mineralization process. One way to reduce the multicollinearity and see the interrelationship of all variables in different dimensions is using a multivariate statistical analysis as the principal component analysis (PCA). Principal component analysis helps to avoid these problems by grouping highly correlated parameters into principal components (PC). Those PCs can be used as a new set of independent variables for regression analysis (Shukla *et al.*, 2004). Nowadays, there are few relevant studies by linking together N mineralization indices, labile organic fractions and soil properties by using multivariate analysis. In addition, this statistical analysis combines variables in such a way that the underlying soil factors relevant for predicting the potential N mineralization might be identified (Ros *et al.*, 2011).

In these soils under no-tillage (NT), POM input by crop residues is highly variable and usually low due to the climatic conditions. On the other hand, regarding the positive effect of POM on nutrient availability; especially, inorganic-N (Galantini and Landriscini, 2007), there is a lack of information about the main factors that regulate the potential N mineralization when they are all considered together under these conditions. In addition, knowing information about the size and the cycling of this inorganic-N pool is important to develop adequate fertilization strategies (Martínez-Lagos *et al.*, 2015).

Our hypothesis is that understanding the interrelationships among the labile SOM fractions, N mineralization indices and soil properties could enable the identification of the main factors affecting the size and kinetics of the mineralizable N pool. The objectives of this study were i) to measure the N mineralization potential; ii) to evaluate mineralization indices and labile organic matter fractions, and iii) to analyze their interrelationship with soil properties for predicting the potential N mineralization in Mollisols under NT of the Argentine Pampas. In this study, PCA coupled with MR was used with a set of N mineralization indices, labile organic fractions, and chemical and physical properties that better explain the variations in the potential N mineralization.

2. Materials and Methods

2.1. Experimental sites

In 2010, 2011 and 2012, composite soil samples (fifteen to twelve soil cylinders) were collected from nine fields under NT. The sites were located in the southwest of the Argentinian Pampas (Figure 1). Predominant soils were Mollisols (Soil Survey Staff, 2010) developed on aeolian sediments (loess),

with a wide range of depth fluctuation, texture, soil organic carbon (SOC) content and fertility (Álvarez and Lavado, 1998). In semiarid and semihumid re-

gions, soils are characterized by a low SOM content, and its dynamics is more strongly affected by water availability (Galantini *et al.*, 2016).

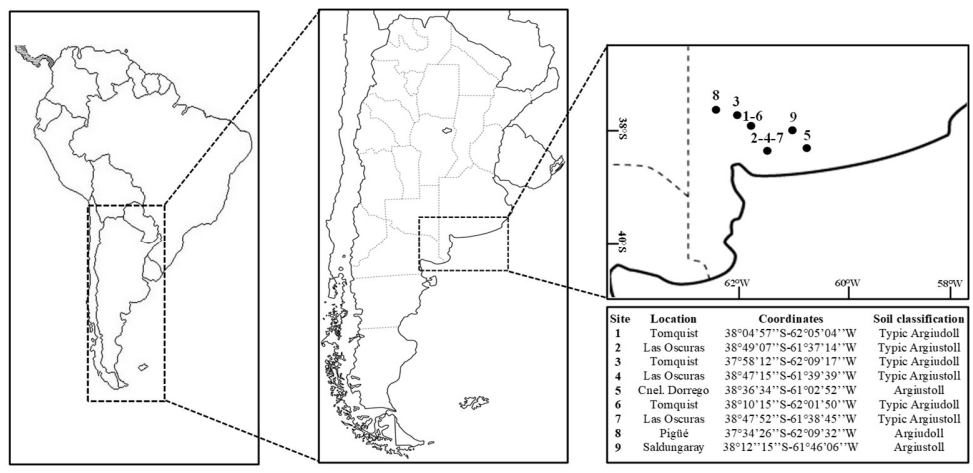


Figure 1. Location of sites in the southwest of the Argentine Pampas.

A composite soil sample (16 and 20 soil cylinders) was collected from N fertilization trials in wheat in the non-fertilized plots before crop seeding at 0-20 cm depth. This sampling depth was selected, because Molisolls have a mollic epipedon or a surface horizon of 18 cm depth (Soil Survey Staff, 2010). The experimental design included three completely randomized blocks. Soil and climate characteristics are shown in Table 1. The rainfall gradient determines an udic (semihumid) soil moisture regime for continental sites (1, 3, 6, 8 and 9) and ustic (semiarid) for sites next to the coast

(2, 4, 5 and 7). Rainfall amount and frequency are irregular across all sites, the rainiest seasons being autumn (March-April) and spring (September-October). All soils had been under continuous agriculture for 10-15 years under NT. The NT system was characterized by the absence of tillage with over 30% residues covering the soil surface in all sites. In general, herbicide (1-2 L ha⁻¹ of glyphosate) was applied for weed control and for initiating the chemical fallow. Data on annual mean temperature and precipitation was collected from SMN (National Weather Service) weather stations.

Table 1. Soil and climate characteristics of sites.

| Sites | Year | Location | Previous crops | Soil classification ^a | MAT ^b (°C) | RA ^c (mm) |
|-------|------|----------------------------|----------------|----------------------------------|--------------------------|-------------------------|
| 1 | 2010 | 38°04'57''S 62°05'04''W | Wheat | Typic Argiudoll | 14.5 | 695 |
| 2 | 2010 | 38°49'07''S 61°37'14''W | Wheat | Typic Argiustoll | 15.0 | 533 |
| 3 | 2010 | 37°58'12''S 62°09'17''W | Barley | Typic Argiudoll | 14.3 | 550 |
| 4 | 2011 | 38°47'15''S 61°39'39''W | Wheat | Typic Argiustoll | 15.0 | 470 |
| 5 | 2011 | 38°36'34''S 61°02'52''W | Barley | Argiustoll | 14.7 | 648 |
| 6 | 2011 | 38°10'15''S 62°01'50''W | Sunflower | Typic Argiudoll | 14.5 | 777 |
| 7 | 2012 | 38°47'52''S 61°38'45''W | Wheat | Typic Argiustoll | 15.0 | 624 |
| 8 | 2012 | 37°34'26''S 62°09'32''W | Soybean | Argiudoll | 13.8 | 1045 |
| 9 | 2012 | 38°12'15''S 61°46'06''W | Wheat | Argiustoll | 14.7 | 694 |

^aSoil Survey Staff (2010). Wheat (*Triticum aestivum* L.); Barley (*Hordeum vulgare* L.); Sunflower (*Helianthus annuus* L.); Soybean (*Glycine Max* L. Merr.). ^bMAT, mean annual temperature. ^cRA, annual rainfall in the sampled year (mm).

2.2. Chemical and physical analyses

The soil samples were air-dried, sieved (<2 mm) and chemically analyzed for the following parameters: SOC by dry combustion using a Leco C automatic analyzer (Leco Corporation, St Joseph, MI); soil organic N (SON) by micro-Kjeldahl method (Bremner, 1996); pH in a soil-water suspension of 1:2.5; Bray-

I extractable P (Pe) (Bray and Kurtz, 1945). Particle size distribution was determined by the pipette method (Gee and Bauder, 1986). Also, undisturbed soil samples were taken to the 0-5, 5-10, 10-15 and 15-20 cm depths by core cylinders 5 cm in height and 4.7 cm in diameter to calculate bulk density (BD) (Blake and Hartge, 1986). The analytical data of the sampled sites are summarized in Table 2.

Table 2. Chemical and physical soil properties at the 0-20 cm depth.

| Sites | SOC (g kg ⁻¹) | SON (g kg ⁻¹) | Pe (mg kg ⁻¹) | pH | C:N | BD (Mg m ⁻³) | Texture | | |
|-------|------------------------------|------------------------------|------------------------------|-----|-----|-----------------------------|---------|------|------|
| | | | | | | | sand | silt | clay |
| 1 | 22 | 1.4 | 20 | 7.0 | 10 | 1.15 | 414 | 380 | 206 |
| 2 | 15 | 1.3 | 26 | 7.4 | 11 | 1.26 | 567 | 399 | 34 |
| 3 | 19 | 1.1 | 15 | 6.7 | 12 | 1.18 | 368 | 404 | 228 |
| 4 | 13 | 1.2 | 19 | 6.2 | 11 | 1.27 | 751 | 154 | 95 |
| 5 | 20 | 1.5 | 22 | 6.2 | 11 | 1.25 | 466 | 321 | 214 |
| 6 | 18 | 1.4 | 14 | 6.4 | 10 | 1.18 | 541 | 284 | 176 |
| 7 | 16 | 1.2 | 26 | 6.0 | 12 | 1.31 | 615 | 177 | 208 |
| 8 | 31 | 2.6 | 10 | 7.6 | 13 | 1.13 | 254 | 538 | 208 |
| 9 | 15 | 1.3 | 10 | 6.3 | 12 | 1.32 | 510 | 266 | 225 |

SOC, soil organic carbon (g kg⁻¹); SON, total soil N (g kg⁻¹); Pe, extractable P (mg kg⁻¹); C:N, carbon: nitrogen ratio of soil; BD, soil bulk density (Mg m⁻³).

2.3. Long-term aerobic incubation

The samples were subjected to an aerobic long-term incubation following the procedure by Griffin *et al.* (2008). Briefly, 250 g of air-dried soil was sieved to 2 mm and placed in 1 L glass jars; two 5 mm diameter holes were drilled into each jar lid to facilitate the aerobic exchange. Distilled water was added to the soil in order to bring their moisture content to 60% of their water-holding capacity (Klute, 1986) and incubated for six drying-rewetting cycles at 25°C. An optimal temperature between 25 and 35°C was determined to study the potential N mineralization, whereas other authors (Griffin *et al.*, 2008; Dessureault-Rompré *et al.*, 2010) concluded that microbial activity was highest at 60% of water-holding capacity and lowest under 30%. Three replicates and a reference sample for controlling soil water content were incubated. Soil water content was measured daily; when it reached 30%, it was rewetted to 60% of water-holding capacity, and another drying-rewetting cycle began. After rewetting, the soils in the jars were sampled with a single 15 mm diameter core for each jar to determine soil inorganic N (NO_3^- -N plus NH_4^+ -N) by steam distillation (Mulvaney, 1996). Following incubation, potential N mineralization was determined by non-linear regression (equation 1), assuming that the cumulative mineralized N (Nmin) fitted with an exponentially first-order kinetic model (Stanford and Smith, 1972):

$$N_{\text{min}} = N_0(1 - e^{-kt})$$

where: Nmin is the cumulative amount of N mineralized at time t, N_0 is the potentially mineralizable N, and k is the rate constant.

The N mineralized in the first two weeks was not used in the curve fitting model because it represented the initial flow of mineralization on rewetting (Nyiraneza *et al.*, 2011) or net mineralization (Ros *et al.*, 2011).

Coarse-textured and fine-textured soils were incubated for 21 and 27 weeks, respectively. Sharifi *et al.* (2007) suggested that incubations should take at least 24 weeks to obtain reliable values of potential N mineralization.

A fixed k value (0.054 week^{-1}) was used in the single exponential model (equation 1) to determine N_{0k} , as proposed by Sharifi *et al.* (2007). Potentially mineralizable N with a fixed constant rate was determined with the same model (equation 1) fitting procedures as N_0 . These authors assumed an average k value determined by Stanford and Smith (1972) for 39 soils, to eliminate the effects of the colinearity of parameters when simultaneously fitting N_0 and k .

2.4. Labile soil organic matter fractions

2.4.1. Particle size fractionation of soil organic matter

The physical fractionation of SOM was performed by wet sieving (Duval *et al.*, 2013). Briefly, 50 g of soil previously air-dried and sieved was dispersed in 120 mL glass containers and mixed with 100 mL of distilled water. Ten glass beads (5 mm in diameter) were added to enhance destruction and reduce potential problems created by different sand contents. After dispersion, the soil suspension was sieved through two connected sieves (105 microns and 53 microns in diameter). The sieves were moved back and forth, and the soil retained in the top sieve was sprinkled with distilled water until the water in the bottom sieve was clear to the naked eye. The fractions obtained were i) a coarse particle size fraction (105-2000 microns) containing coarse sands and coarse POM, and ii) a medium particle size fraction (53-105 microns) containing more stable or fine POM, and fine and very fine sand; the fine particle size fraction (<53 microns) was discarded. Each particle size fraction was analyzed for C and N, and two fractions were obtained: i) particulate organic

C and N in the coarse particle size fraction (cPOM-C and cPOM-N, respectively) and ii) particulate organic C and N in the medium particle size fraction (fPOM-C and fPOM-N, respectively). Carbon and N contents in POM were determined using the same methods as for SOC and SON, respectively.

2.4.2. Total and soluble carbohydrates

Extraction of carbohydrates was performed employing two different samples. Total carbohydrates (CHt) extraction was performed by an acid hydrolysis as follow: 1 g of soil sample was treated with 10 mL $0.5 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$, heated at 80°C for 24 h. In the other sample, soluble carbohydrates (CHs) extraction was carried out as follow: 1 g of soil sample was suspended in 10 mL of distilled water and heated at 80°C for 24 h. After the extraction with hot water, H_2SO_4 was added to obtain a 0.5 mol L^{-1} concentration as in the dilute acid hydrolysis procedure, and immediately processed. After extraction by each way, each suspension was centrifuged at 4000 rpm during 15 min (Puget *et al.*, 1999). Carbohydrate contents of the extract were determined by spectrometry using the sulphuric-phenol spectrometric method with glucose as the standard (Dubois *et al.*, 1956).

2.5. Indices of N mineralization

2.5.1. Chemical hydrolyzable nitrogen

The hydrolyzable N was chemically extracted by soil digestion with a strong salt solution of $2 \text{ mol L}^{-1} \text{ KCl}$, as described by Gianello and Bremner (1986). The procedure was performed by digesting 3 g of soil in 20 mL of the solution at 100°C for 4 hours in a block digester. The sample was then cooled and $\text{NH}_4^+\text{-N}$ was determined by steam distillation (Mulvaney, 1996). The initial soil $\text{NH}_4^+\text{-N}$ was extracted at room temper-

ature and the hydrolyzable N (Nhyd) was determined by subtracting the initial $\text{NH}_4^+\text{-N}$ without digestion.

2.5.2. Cold nitrate-extraction

Briefly, 5 g of air-dried soil was added to 50 mL of $2 \text{ mol L}^{-1} \text{ KCl}$. The suspension was shaken for 30 min at 290 rpm. The samples were then centrifuged and the extract was filtered. The $\text{NO}_3\text{-N}$ concentration was determined by steam distillation (Mulvaney, 1996).

2.5.3. Determination by UV-visible spectrophotometry

The procedure was performed according to the method described by MacLean (1964). A 5 g of soil was added to 100 mL of $0.01 \text{ mol L}^{-1} \text{ NaCO}_3\text{H}$. The suspension was shaken for 15 min in a 250 mL Erlenmeyer flask. The samples were centrifuged and the suspensions were filtered through a Whatman grade 42 filter paper. The ultraviolet absorption at $205(\text{N}_{205})$ and $260(\text{N}_{260}) \text{ nm}$ was measured with a T60U UV-visible spectrophotometer (PG Instruments). When UV measurements were made at 205 nm, two drops of concentrated HCl were added to remove carbonate wavelength peaks.

2.5.4. Anaerobic nitrogen

Anaerobic N (Nan) was determined following the method by Martínez *et al.* (2017) in a short-term anaerobic incubation. A 5 g of soil was put into a test tube and 25 mL of distilled was added. The tube caps were securely tightened and then the tubes were incubated for 7 days at 40°C under anoxic conditions. After incubation, the samples were transferred to a distillation flask and 25 mL of $4 \text{ mol L}^{-1} \text{ KCl}$ was added; the $\text{NH}_4^+\text{-N}$ was determined by steam distillation (Mulvaney, 1996). The Nan content was estimated by subtracting

inorganic-N in the form of NH_4^+ -N extracted with 2 mol L^{-1} KCl -at room temperature and without incubation- from the amount obtained in the incubated extract.

2.5.5. Net N mineralization

The net N mineralization (gNmin) involved pre-incubation of soils using the long-term aerobic method (Sharifi *et al.*, 2007). The air-dried soil was placed in the respective bottle and distilled water was added to 60% of water-holding capacity; this was followed by incubation at 25°C for 12 days. The jars were then sampled with a sampler, and inorganic N (NO_3^- -N plus NH_4^+ -N) was determined by steam distillation (Mulvaney, 1996).

2.6. Statistical analysis

A non-linear regression analysis was used to obtain N_0 , N_{okf} and k of equation 1. Pearson's correlations were performed between N_0 and N_{okf} with soil N mineralization indices, labile organic fractions and soil properties to evaluate the relationships between individual variables. A PCA was performed with the significant correlated ($P < 0.05$) variables for N_0 and N_{okf} . The objective of multivariate analysis was to analyze all the variables correlated together and the relationship among them, and how they affected the potential N mineralization pool. Also, this analysis was employed as a data-reduction tool to select the most appropriate factors, through which the number of independent variables could be reduced and problems related to multicollinearity could be eliminated (Li *et al.*, 2013). Only PCs with eigenvalues > 1 were retained for the regression analysis, because they explained the data variability. Within each PC, variables receiving weighted loading values within 10%

of the highest weighted loading were selected for each PC (Li *et al.*, 2013). Multiple regressions were performed using the $\text{PC} > 1$ by multivariate analysis for predicting N_0 and N_{okf} (dependent variables) using the stepwise model with a maximum P-value of 0.05 for input and output. The MR model was used to determine the best combination of PC that maximizes the prediction of N_0 and N_{okf} . The statistical analysis was carried out with Infostat software (Di Rienzo *et al.*, 2013).

3. Results

3.1. Soil chemical and physical characteristics

Soil organic C ranged from 13 g kg^{-1} to 31 g kg^{-1} with a mean value of 19 g kg^{-1} (Table 2). Soil pH was slightly alkaline to mildly acid with an average of 7.0 and a range between 6.0 and 7.6. Regarding soil texture, sand content in soil was between 254 and 751 g kg^{-1} , averaging 498 g kg^{-1} . Taking into account the amount of fine fractions (silt plus clay), whose mean values were 502 g kg^{-1} , a significant and positive relationship was found between silt plus clay and SOC (data not shown), with a high coefficient of determination ($R^2 = 0.76$).

3.2. Potential N mineralization

A broad range of values was found for N_0 and N_{okf} , the highest being 220.7 and 172.4 mg kg^{-1} , and the lowest 29.9 and 26.9 mg kg^{-1} , respectively. The mean value of k across all sites was 0.0329 week^{-1} , ranging from 0.0125 to 0.163 week^{-1} (Table 3). The relationship between N_0 and N_{okf} was found to be very close ($y = 1.19x + 0.97$; $P < 0.001$) with a high coefficient of determination ($R^2 = 0.94$) (data not shown).

Table 3. Potentially mineralizable N in the long term at a variable and at a fixed constant rate, and mineralization constant rates (mean \pm standar deviation) by site.

| Sites | N_0 | N_{0kf} | k |
|-----------------|------------------------|-----------------|-----------------------|
| | (mg kg ⁻¹) | | (week ⁻¹) |
| 1 | 153.3 \pm 17.9 | 130.8 \pm 8.6 | 0.024 \pm 0.006 |
| 2 | 109.4 \pm 7.9 | 78.3 \pm 4.4 | 0.017 \pm 0.004 |
| 3 | 139.0 \pm 6.7 | 133.8 \pm 1.2 | 0.044 \pm 0.015 |
| 4 | 29.9 \pm 3.4 | 26.9 \pm 2.9 | 0.031 \pm 0.006 |
| 5 | 130.3 \pm 15.1 | 111.8 \pm 2.3 | 0.030 \pm 0.011 |
| 6 | 117.5 \pm 11.2 | 89.5 \pm 3.6 | 0.018 \pm 0.005 |
| 7 | 50.0 \pm 6.4 | 49.8 \pm 2.7 | 0.094 \pm 0.065 |
| 8 | 215.3 \pm 5.0 | 172.4 \pm 2.8 | 0.019 \pm 0.001 |
| 9 | 113.2 \pm 9.4 | 87.8 \pm 5.0 | 0.019 \pm 0.003 |
| mean | 117.5 | 97.9 | 0.033 |
| SD ^a | 53.1 | 43.1 | 0.030 |
| CV ^b | 45 | 44 | 92 |

N_0 , aerobic potentially mineralizable N; N_{0kf} , potentially mineralizable N at a fixed constant rate (k) (0.054 week⁻¹); k , mineralization constant rate (week⁻¹). ^aSD, standard deviation; ^bCV, coefficient of variation.

3.3. Soil N-mineralization indices and soil organic matter fractions

Nitrogen mineralization indices and soil labile organic fractions are shown in Table 4. Regarding biological methods, the mean gNmin value for all sites was 64.2 mg kg⁻¹, ranging from 30.3 to 116.3 mg kg⁻¹. Nan ranged from 25.2 to 99.4 mg kg⁻¹, whereas, Nhyd ranged from 10.7 to 67.2 mg kg⁻¹. The mean NO₃-N value was 20.1 mg kg⁻¹, with a range between 11.2 mg kg⁻¹ and 27.5 mg kg⁻¹. The mean UV-absorbance values were 1.84 and 0.44 for N₂₀₅ and N₂₆₀, respectively. The CV between mineralization indices followed the sequence N₂₆₀<

N₂₀₅< NO₃-N <gNmin <Nan <Nhyd, where values ranged from 16 to 40%.

The mean values of labile organic fractions were 1.07; 1.39; 0.103 and 0.161 g kg⁻¹ for cPOM-C, fPOM-C, cPOM-N and fPOM-N, respectively (Table 4). Considering POM-C as the sum of cPOM-C and fPOM-C, it represented about 15.6% of SOC, whereas POM-N (cPOM-N+fPOM-N) accounted for about 18.9% of SON. Total carbohydrates ranged from 2.09 to 3.41 g kg⁻¹, averaging 2.62 g kg⁻¹; CHs ranged from 0.22 to 0.44 g kg⁻¹, with an average of 0.29 g kg⁻¹. Total carbohydrates represented about 16.9% of SOC, and CHs about 1.8%. Labile organic fractions showed a low CV, from 7 to 28%.

Table 4. Nitrogen mineralization indices and labile soil organic fractions (mean ± standard deviation) by site.

| Sites | Mineralization indices | | | | | | Labile organic matter fractions | | | | | |
|-----------------|------------------------|----------|----------|--------------------|------------------|------------------|---------------------------------|-----------|------------|-------------|-----------|-----------|
| | gNmin | Nan | Nhyd | NO ₃ -N | N ₂₀₅ | N ₂₆₀ | cPOM-C | fPOM-C | cPOM-N | fPOM-N | CHt | CHs |
| | (mg kg ⁻¹) | | | | (absorbance) | | (g kg ⁻¹) | | | | | |
| 1 | 47.4±11 | 41.5±4.5 | 67.2±2.8 | 11.2±1.9 | 1.58±0.03 | 0.48±0.05 | 1.54±0.13 | 2.26±0.14 | 0.126±0.01 | 0.188±0.01 | 3.39±0.23 | 0.51±0.04 |
| 2 | 48.3±4.3 | 42.5±11 | 34.5±9.0 | 23.3±7.1 | 1.53±0.35 | 0.38±0.06 | 1.20±0.39 | 0.76±0.14 | 0.083±0.01 | 0.104±0.03 | 2.29±0.24 | 0.27±0.02 |
| 3 | 60.6±16 | 25.2±5.6 | 10.7±2.1 | 27.5±2.7 | 2.28±0.35 | 0.45±0.07 | 0.83±0.13 | 1.64±0.41 | 0.096±0.01 | 0.176±0.01 | 2.62±0.06 | 0.30±0.03 |
| 4 | 30.3±5.1 | 32.2±1.4 | 25.7±2.4 | 14.0±3.5 | 1.45±0.09 | 0.39±0.07 | 0.73±0.06 | 1.15±0.81 | 0.122±0.01 | 0.134±0.02 | 1.97±0.23 | 0.30±0.01 |
| 5 | 62.7±6.3 | 61.1±3.5 | 34.1±6.6 | 17.7±8.5 | 1.71±0.08 | 0.51±0.09 | 1.34±0.47 | 1.83±0.93 | 0.106±0.02 | 0.179±0.01 | 2.29±0.23 | 0.39±0.03 |
| 6 | 67.1±11 | 99.4±4.8 | 36.9±13 | 18.2±4.9 | 1.58±0.03 | 0.48±0.06 | 1.65±0.25 | 0.95±0.08 | 0.152±0.04 | 0.161±0.01 | 3.17±0.35 | 0.41±0.05 |
| 7 | 88.4±6.0 | 27.1±0.8 | 25.2±4.8 | 20.3±9.8 | 1.85±0.24 | 0.44±0.03 | 0.62±0.12 | 0.55±0.03 | 0.052±0.01 | 0.081±0.004 | 2.18±0.28 | 0.27±0.01 |
| 8 | 116.3±15 | 70.9±4.5 | 47.1±7.7 | 23.1±7.0 | 2.29±0.41 | 0.38±0.04 | 1.01±0.17 | 2.40±0.89 | 0.102±0.01 | 0.256±0.01 | 4.28±0.56 | 0.75±0.07 |
| 9 | 57.1±16 | 74.2±5.0 | 46.2±6.4 | 25.2±0.7 | 2.28±0.35 | 0.45±0.07 | 0.70±0.08 | 0.95±0.06 | 0.087±0.03 | 0.166±0.01 | 2.22±0.43 | 0.38±0.01 |
| mean | 64.2 | 52.7 | 36.4 | 20.1 | 1.84 | 0.44 | 1.07 | 1.39 | 0.103 | 0.161 | 2.72 | 0.40 |
| CV ^a | 40 | 47 | 49 | 35 | 22 | 16 | 19 | 28 | 15 | 7 | 11 | 8 |

^aCV, coefficient of variation. gNmin, Net N mineralization (mg kg⁻¹); Nan, anaerobic N (mg kg⁻¹); Nhyd, chemical labile N minus initial N in ammonium form (mg kg⁻¹); N-NO₃, nitrate N (mg kg⁻¹); N₂₀₅, N determined by spectrometer at 205 nm (absorbance); N₂₆₀, N determined by spectrometer at 260 nm (absorbance); cPOM-C, coarse particulate organic carbon (g kg⁻¹); cPOM-N, coarse particulate organic nitrogen (g kg⁻¹); POM-C, fine particulate organic carbon (g kg⁻¹); fPOM-N, fine particulate organic nitrogen (g kg⁻¹); CHt, total carbohydrates (g kg⁻¹); CHs, soluble carbohydrates (g kg⁻¹).

3.4.. *Relationships among N_0 and N_{0kf} with N-mineralization indices, labile organic fractions and soil properties*

On the basis of Pearson's correlations (Table 5), high positive and significant relationships were detected between N_0 and N_{0kf} with some labile organic fractions ($r>0.64$), *i.e.* C and N in fine POM fractions (fPOM-C and fPOM-N) and soil carbohydrates (CHt and CHs). Also, some significant correlations were observed between N_0 and N_{0kf} with N-mineralization indices, but with a low correlation coefficient ($r<0.53$). The N_{0kf} showed positive and significant relationships with

gNmin, SOC, SON and its fractions. As to the indices determined by UV, N_{205} showed significant relations with N_0 and N_{0kf} with a low correlation coefficient ($r<0.50$).

Considering soil properties, close correlations were found between N_0 at a variable and at a fixed constant rate with most soil chemical and physical properties, especially with texture (sand and silt content) (Table 5). Regarding soil texture, high and negative correlations were found between N_0 and N_{0kf} with sand, and positive with silt, which shows that these soils have a low clay content, except for soil 8 (Table 2).

Table 5. Pearson's correlation of potentially mineralizable N at a variable and at a fixed rate with N mineralization indices, labile organic matter fractions and soil properties.

| | N ₀ | | N _{0kf} | |
|--------------------|----------------|---------|------------------|---------|
| | r | p-value | r | p-value |
| gNmin | 0.53 | ** | 0.50 | ** |
| Nan | 0.39 | * | 0.25 | ns |
| Nhyd | 0.40 | * | 0.31 | ns |
| NO ₃ -N | 0.21 | ns | 0.19 | ns |
| N ₂₀₅ | 0.43 | * | 0.47 | * |
| N ₂₆₀ | 0.04 | ns | 0.11 | ns |
| cPOM-C | 0.33 | ns | 0.30 | ns |
| fPOM-C | 0.64 | *** | 0.68 | *** |
| cPOM-N | 0.17 | ns | 0.13 | ns |
| fPOM-N | 0.83 | *** | 0.84 | *** |
| CHt | 0.72 | *** | 0.72 | *** |
| CHs | 0.71 | *** | 0.69 | *** |
| SOC | 0.71 | *** | 0.67 | ** |
| SON | 0.75 | *** | 0.68 | *** |
| pH | 0.71 | *** | 0.64 | *** |
| Pe | -0.50 | * | -0.48 | * |
| C:N | 0.25 | ns | 0.28 | ns |
| BD | -0.75 | *** | -0.61 | *** |
| sand | -0.89 | *** | -0.92 | *** |
| silt | 0.85 | *** | 0.89 | *** |
| clay | 0.39 | * | 0.51 | ** |

r, correlation coefficient; N₀, aerobic potentially mineralizable N; N_{0kf}, potentially mineralizable N at a fixed constant rate (*k*) (0.054 week⁻¹). See abbreviations in Table 2 and 4. *, **, ***, significant correlation at 0.05; 0.01, 0.001 probability levels, respectively. ns, no significant.

3.5. Grouping N-mineralization indices, labile organic fractions and soil properties

Considering the significant highly correlated variables for N₀ and N_{0kf} (Table 5), we conducted a PCA (Table 6). In N₀, the PCA showed eigenvalues greater than 1 in the first four PCs, which accounted for 82% of the total variance. The PC1 explained 51% of the variance, and the loading was positive with SOM fractions (SOC, SON, CHs, fPOM-N) and negative with sand (Table 6). PC2 explained 14% of the variance, in which clay content showed positive associations. The third PC accounted for 10% and

it had a high and positive loading for Nan, whereas the fourth PC explained 7%, with a high and negative loading for cPOM-N. In N_{0kf}, the PCA showed eigenvalues greater than 1 in the first three PCs. The PC1 explained 71% of the variance and had the same variables for N₀ (Table 6). The PC2 accounted for 15% of the variance, in which clay content showed positive associations as in PCA for N₀. The third PC represented 9% and had high and positive loading for gNmin. In fact, it can be observed that the first three PCs are divided in terms of their association with the size of SOM fractions (PC1), with soil texture (PC2) and with the N lability (PC3).

Table 6. Results of principal component analysis for N_0 and N_{0kf}

| | N_0 | | | | N_{0kf} | | |
|------------------------|--------------|-------------|-------------|--------------|--------------|-------------|-------------|
| | PC1 | PC2 | PC3 | PC4 | PC1 | PC2 | PC3 |
| Eigenvalue | 8.13 | 2.17 | 1.55 | 1.16 | 7.80 | 2.09 | 1.30 |
| Proportion of variance | 0.51 | 0.14 | 0.10 | 0.07 | 0.56 | 0.15 | 0.09 |
| Total variance | 0.51 | 0.65 | 0.75 | 0.82 | 0.56 | 0.71 | 0.80 |
| Variable | Eigenvectors | | | | Eigenvectors | | |
| gNmin | 0.21 | 0.25 | -0.32 | 0.30 | 0.22 | 0.24 | 0.46 |
| Nan | 0.15 | 0.14 | 0.48 | 0.42 | - | - | - |
| Nhyd | 0.16 | -0.18 | 0.37 | -0.04 | - | - | - |
| N ₂₀₅ | 0.16 | 0.43 | -0.35 | -0.05 | 0.17 | 0.45 | 0.33 |
| cPOM-C | - | - | - | - | - | - | - |
| fPOM-C | 0.25 | -0.11 | 0.01 | -0.47 | 0.26 | -0.07 | -0.26 |
| fPOM-N | 0.33 | 0.05 | 0.13 | -0.15 | 0.33 | 0.05 | -0.23 |
| CHt | 0.28 | 0.003 | 0.28 | -0.07 | 0.28 | -0.01 | -0.35 |
| CHs | 0.30 | -0.09 | 0.20 | -0.05 | 0.31 | -0.08 | -0.24 |
| SOC | 0.30 | 0.01 | -0.16 | 0.29 | 0.31 | -0.003 | 0.26 |
| SON | 0.31 | -0.04 | -0.07 | 0.31 | 0.32 | -0.06 | 0.19 |
| Pe | -0.22 | -0.30 | -0.24 | -0.09 | -0.22 | -0.29 | 0.21 |
| pH | 0.23 | -0.40 | -0.27 | 0.14 | 0.23 | -0.41 | 0.30 |
| Sand | -0.31 | -0.07 | 0.19 | 0.19 | -0.32 | -0.07 | -0.06 |
| Silt | 0.29 | -0.19 | -0.27 | -0.02 | 0.29 | -0.21 | 0.20 |
| Clay | 0.15 | 0.49 | 0.08 | -0.38 | 0.15 | 0.53 | -0.24 |
| BD | -0.23 | 0.37 | -0.05 | 0.16 | -0.24 | 0.38 | 0.19 |

Bold letter indicates the variables with higher loadings. N_0 , aerobic potentially mineralizable N; N_{0kf} , potentially mineralizable N at a fixed constant rate (0.054 week⁻¹). See abbreviations in Table 2 and 4.

3.6. Prediction of N mineralizable using multivariate analysis

Taking into account the new variables generated by the PCA (Table 6), the MR using stepwise meth-

od selected one PC for N_0 at a variable and at a fixed constant rate (Table 7). High R^2 values were found for predicting N_0 (adj $R^2=0.84$) and N_{0kf} (adj $R^2=0.83$).

Table 7. Prediction of potentially mineralizable nitrogen at a variable and at a fixed constant rate using principal component as regressor variable.

| Dependent variable | Regressor variable | Estimated parameter | Standard error | P-value | 95% Confidence limits | | R^2 | Adj R^2 |
|--------------------|--------------------|---------------------|----------------|---------|-----------------------|-------|-------|-----------|
| N_0 | Intercept | 117.5 | 4.05 | <0.0001 | 109.2 | 125.9 | 0.85 | 0.84 |
| | PC1 N_0 | 17.1 | 1.5 | <0.0001 | 14.8 | 20.1 | | |
| N_{0kf} | Intercept | 97.9 | 3.4 | <0.0001 | 90.9 | 104.9 | 0.84 | 0.83 |
| | PC1 N_{0kf} | 14.5 | 1.3 | <0.0001 | 11.8 | 17.1 | | |

All prediction equations are constructed in the form $y = \beta_0 + x_1\beta_1$, where β_0 is the intercept and β_1 is the parameter estimates and x_1 is the new variable generated by PCA. N_0 , aerobic potentially mineralizable N; N_{0kf} , potentially mineralizable N at a fixed constant rate (k) (0.054 week⁻¹).

4. Discussion

4.1. Potential N mineralization, soil N-mineralization indices and labile SOM fractions

Values of N_0 were similar to those reported by other authors (Sharifi *et al.*, 2007; Nyiraneza *et al.*, 2011). Using a fixed k (0.054 wk^{-1}) to determine N_{0kf} produced slightly lower values compared with N_0 . Although use of the fixed k approach reduced the CV of potential N mineralization in other studies (Sharifi *et al.*, 2007), in the present study the CV of N_0 and N_{0kf} was similar.

The mean value of k was closer to the 0.054 week^{-1} reported by Stanford and Smith (1972); this difference among soils was also observed by other authors (Sharifi *et al.*, 2007). The k values were greater in sandy soils than in loam soils. Schomberg *et al.* (2009) found higher k values in medium- to coarse-textured soils than in fine-textured soils. This may have occurred because SOM is physically less protected by clays (Nyiraneza *et al.*, 2011) and by the higher labile fraction in medium- to coarse-textured soils (Martínez and Galantini, 2017). The high relationship ($R^2=0.94$) between N_0 and N_{0kf} showed that potential mineralization can be measured more easily by standardizing k at a fixed value, despite contrasting k values observed in Table 3 and the differences in soil properties. In addition, other authors (Schomberg *et al.*, 2009; Dessureault-Rompré *et al.*, 2010) stated that it is difficult to identify a compositional factor that is predictive of k .

The mean gNmin value was twice as much as that reported by Schomberg *et al.* (2009), who obtained 21 mg kg^{-1} in different soils in Canada and the USA under contrasting managements. However, they fit in the range studied by Curtin and Wen (1999) for soils under different managements in Canada and they coincide with studies by Dessureault-Rompré *et al.* (2010), who studied several soils from four eco-zones

in the same country. The Nan values were within the range reported by other authors (Sharifi *et al.*, 2007; Schomberg *et al.*, 2009). The Nhyd values were similar to those obtained by Gianello and Bremner (1986) for 33 tropical soils, but higher than those ($2.3\text{--}16.1 \text{ mg kg}^{-1}$) reported by Schomberg *et al.* (2009). Ranges for N_{205} and N_{260} resembled those reported by Sharifi *et al.* (2007) in soils at 0–15 cm under different crops in contrasting climate areas. On the other hand, high variability (relatively high CV) in mineralization indices –except for N_{205} and N_{260} – highlighted the differences among soil parameters in these Mollisols. Also, Sharifi *et al.* (2007) reported low CV for N_{205} and N_{260} when comparing mineralization methods in various soils.

In contrast, differences across sites are very few in terms of labile organic fractions because of a low CV. They also showed that the labile organic fractions that make up POM represent a small part of SOM.

4.2. Relationships among N_0 , N_{0kf} , N-mineralization indices and labile organic fractions and soil properties

On the basis of Pearson's correlations (Table 5), low significant relationships were detected between N_0 and Nan ($P<0.05$), as reported by other authors (Sharifi *et al.*, 2007). The positive relationship between N_0 and Nhyd was similar to the one found by Curtin and Wen (1999) in cultivated soils of Canada, and they reported an $R^2=0.13$ for the 0–15 cm depth. Correlation between N_0 and gNmin was similar to that observed by Nyiraneza *et al.* (2011), who reported an r of 0.63 in humid areas under conventional tillage (CT) at the 0–7.5 cm depth. The N_{0kf} showed positive significant relations with gNmin, SOC, SON and its fractions. As to the indices determined by UV, N_{205} showed significant relations with N_0 and N_{0kf} . The most important variables related to N_0 and N_{0kf} were the labile organic fractions ($r>0.64$), *i.e.* C and N in fine particulate

organic fractions (fPOM-C and fPOM-N) and soil CH (CHt and CHs). With respect to this latter, it is important to emphasize its capacity in providing energy to soil microorganisms, given that CH content is enhanced under NT (Duval *et al.*, 2013). In general, labile organic matter (*e.g.* fPOM-C; fPOM-N; CHt and CHs) tended to be more strongly related to N_0 and N_{okf} than to N mineralization indices.

4.3. Prediction of N mineralizable using multivariate analysis

For both predictions the results indicate that N mineralization indices are not relevant as were the organic fractions and soil texture. The results showed that both total and labile organic fractions and sand content are in the same PC. The best prediction was given by SOC, SON, CHs, fPOM-N and sand content. The importance of these SOM fractions for N mineralization has been further corroborated by numerous studies evaluating the predictive value of single soil organic fraction for the prediction of mineralizable N (Ros *et al.*, 2011; Martínez *et al.*, 2017). Also, these organic fractions are known to be highly labile (Duval *et al.*, 2013; Duval *et al.*, 2016) and they can release nutrients in the short term (Galantini and Landriscini, 2007). Other authors (Ros *et al.*, 2011) had reported close relationships between CHs and mineralizable N. Duval *et al.* (2013) suggested that CHs are mainly of microbial origin, considering them as highly labile component as energy source for soil microorganisms. In addition, Haynes (2005) stated that hot water-extracted organic matter was largely composed of polysaccharides and N-containing compounds as amino-N and amides. Therefore, CHs and fPOM-N could function as a source of N within the mineralization in these Mollisols. Hence, both labile organic fractions could be used as N mineralization indices, especially when assessing soil quality in these Mollisols characterized

by low POM input from crop residues. Several authors (Puget *et al.*, 1999; Duval *et al.*, 2013; Duval *et al.*, 2016) had already discussed about the high sensitivity of these labile fractions under different land managements.

Regarding soil texture, Martínez and Galantini (2017) showed that N release was faster in sandy soils than in clayey soils because the physical protection of SOM was lower. In this study, sand regulated the levels of SOM and its labile fractions; *i.e.* soils with a higher sand content have lower levels of SOC, SON and its labile fractions, resulting in lower N_0 and N_{okf} . This could be verified by highly significant and negative correlations between sand with SOC ($r = -0.67$), SON ($r = -0.67$), CHs ($r = -0.63$) and fPOM-N ($r = -0.80$) (data not shown). These results suggest that N mineralization in these soils was related to the size of SOM and its fractions rather than to its quality. These results were similar to the findings of other studies in contrasting soils (Ros *et al.*, 2011).

Results of the current study suggest that potential N mineralization -considering a variable and a fixed constant rate - may be mainly controlled by the variables related to the size of SOM and some labile organic fractions related to the C- and N-cycling (fPOM-N and CHs), despite the low POM input from residues in these soils. This study helps to better understand the complex dynamics of soil N mineralization in these Mollisols. However, the N mineralization is a dynamic process that can vary during the crop growing season as a result of the interaction between labile organic fractions and water availability, which are highly variable factors in these areas. The results of this study highlight about the factors that controls the size and kinetics of potential N mineralizable to better predict growing season N mineralization. Hence, the labile pools of soil organic N should be studied in further detail over the crop growing season.

Conclusions

The results of this study concluded that in Mollisols under these conditions no single N mineralization index, labile organic fraction or soil property can accurately predict N_0 at a variable or at a fixed constant rate. In spite of this result, it is important to emphasize that soil sand content is the most influential predictor of potential N mineralization. Furthermore, a constant rate value (0.054 week^{-1}) can be used to estimate the N to be mineralized during the crop growing season with similar accuracy. The multivariate analysis such as PCA allowed combination of mineralization indices, labile organic fractions and soil variables into groups relevant enough for determining the mineralizable N pool. Also, it improved the prediction of N_0 and N_{0kf} by using PC as regressor and it allowed elimination of the multicollinearity generated from MR models using high correlated variables. The mineralization N pool may be controlled mainly by the variables related to the size of SOM and some fractions in these Mollisols, such as SOC, SON, CHs and fPOM-N. These latter labile organic fractions would be expected to be more sensitive to short-term changes in potential N mineralization due to changes in soil management practices and history. However, its effectiveness for other soils and tillage conditions has to be evaluated.

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