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Review Article

Critical Review on the Inclusion of the Measurement of Nitrogen Produced in Short Incubations (Nan) in the Diagnostic Protocol of Nitrogenous Fertility in Cordoba (Argentina)

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Abstract: N is the nutrient that is applied in the greatest quantity to increase crop yields. Having a soil analysis that allows estimating the N needs of the crop would have economic and environmental benefits. The measurement of total C, total N and the content of N-nitrates are part of the analytical protocol that is carried out in pre-sowing for diagnostic purposes. However, the relationship between these measurements and actual N mineralization in the field is only approximate. To improve the estimation of N availability, it is necessary to consider the initial inorganic N and the mineralized N (Nmin) during the growing season. The search for an accurate predictor of Nmin that can be done quickly in the laboratory has sparked interest in measuring N in short anaerobic incubations or Nan. In this article, the soil properties that intervene in the N mineralization process that affect the measurement of Nan are exposed to know the scope: advantages and disadvantages of the inclusion of Nan in the routine protocol for diagnostic purposes for its use as a predictor of mineralized N during the crop cycle.

Keywords: Diagnosis, nitrogen, technique, interpretation.

INTRODUCTION

In Argentina, the analytical methodology used for the diagnosis of nitrogenous fertility in wheat crops is based on the determination of the N content of nitrates ($N\text{-}NO_3^-$) in pre-sowing from 0 to 60 cm (Barbieri, Echeverría and Sainz Rozas, 2012). In maize, the $N\text{-}NO_3^-$ content is measured from 0 to 30 cm in the V6 stage (Esposito, Castillo and Balboa, 2006).

These diagnostic methods do not consider the N released during growth season by organic N (Norg) mineralization. To improve the estimation of the dose of N to be applied, it is necessary to consider both sources of N: initial inorganic N and mineralized N (Nmin) (Reussi Calvo *et al.*, 2018).

The procedure to obtain the fraction of Norg that corresponds to potentially mineralizable N (NPM) is still controversial (Wang, Smith, Chalk & Chen, 2001).

Various mathematical models have been tested to estimate Nmin, most of which have only achieved slight progress in the search for a "universally" acceptable method (Galvis-Spinola and Hernández-Mendoza, 2004).

The use of long incubations (32 weeks) proposed by Stanford and Smith (1972) to know the NPM is not practical because the diagnosis of nitrogenous fertility must be carried out a few days before planting, which is why this type of test does not it is carried out in soil laboratories for diagnostic purposes (Echeverria, San Martin and Bergonzi, 2000).

In order for it to be possible to evaluate the contribution by mineralization from a soil analysis, it is necessary to include in the diagnostic protocol a simple, fast and low-cost analytical method that allows a reliable estimate to be made.

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Genovese, Echeverria, Studdert and Sainz Rozas, 2009).

The soil laboratories of the Pampas region (humid and sub-humid) promote the measurement of the N produced in short anaerobic incubations or Nan to know the Nmin (Fertilab, 2014).

Studies carried out in the Pampas region show that Nan as a laboratory method for diagnostic purposes has had various levels of success as a predictor of Nmin (Martínez, Duval and Galantini, 2018; Orcellet *et al.*, 2017; Melchiori and Gudelj, 2010; Reussi Calvo *et al.*, 2013; Reussi Calvo *et al.*, 2014; Reussi Calvo *et al.*, 2018).

During the last agricultural campaigns, the interest of the technicians and producers of the extra Pampa region has increased so that the soil laboratories include the Nan measurement in the pre-planting analyzes for diagnostic purposes.

However, as pointed out by Martínez *et al.*, (2018) there is not enough information on the potentiality of Nan for its use as an indicator of N mineralization in extra Pampas (Argentina) with water limitations. In these environments (arid and semi-arid), the mineralization process occurs in pulses, in a heterogeneous manner, in response to microclimate conditions and the contribution of organic matter (Bachmeier and Rollán, 2004; Celaya-Michel and Castellanos-Villegas, 2011). Under these conditions it is difficult to find a simple method that can be used to predict the net effect of several concurrent processes that regulate the mineralization process (Dominguez, Studdert, Echeverría, and Andrade, 2001).

There are several aspects to consider so that the information resulting from the Nan measurement can be used effectively as a tool with diagnostic criteria.

This article analyzes the factors inherent to the N mineralization process (cultural management of production batches) that affect the results of the measurement of Nan and considers the operational aspects in the field and in the laboratory to be defined for the inclusion of Nan as a routine analytical technique for diagnostic purposes.

N Mineralization

The initial availability and subsequent supply of N respond to complex relationships between the factors that affect organic N mineralization and mineral N immobilization (Walley, Yates, van Groenigen, and van Kessel, 2002). The direction, magnitude and result of such relationships are determined by environmental conditions and are finally defined by the influence of management decisions such as tillage, quantity and quality of residues returned to the soil and the contribution of N by fertilization (Studdert, Carabaca, and Echeverría, 2000).

In not tillage (SD), the presence of stubble on the surface causes an increase in the content of organic C and N that accumulate from 0 to 5 cm deep due to the lower rate of decomposition given the absence of mechanical mixing (Meyer, Frigerio and Cortes, 2012; Koritko *et al.*, 2019, Rollán and Bachmeier, 2015) which is why the measured values of Nan are significantly higher in the upper stratum (Fabrizzi, Morón, García, 2003; Rodríguez, Videla, Zamuner, Picone, Pose *et al.*, 2015, Domínguez *et al.*, 2016; Soon, Haq y Arshad , 2007; Martínez, Galantini, Duval, y López 2015).

Due to the stratification generated by the SD, the magnitude of the analytical result of the Nan measurement will depend largely on the thickness analysed and that its predictive capacity of the Nmin will be regulated by the way in which the soil-plant-environment relationships affect the mineralization-immobilization processes that occur at the surface level during the crop cycle (Steinbach, Alvarez and Valente, 2004) which affects the sampling protocol and the interpretation of the results.

Sampling Protocol

Among the aspects to consider regarding the sampling protocol to be followed in the environments of the province of Córdoba are the criteria to be followed for taking a representative sample, the type of sample, the sampling section and the number of subsamples per sample.

The published works agree on the use of composite samples (cs) for the Nan measurement and differ in the number of subsamples per mc from a minimum of 5 subsamples per cs (Dominquez *et al.*, 2016) to a maximum of 20 subsamples per cs (Benintende *et al.*, 2007). Others do not specify the number of subsamples used (Calviño and Echeverria, 2003; Echeverria San Martin and Bergonzi, 2000; Martínez, Duval and Galantini, 2018). There are no studies available that indicate the spatial variability associated with the Nan measurement, which would allow estimating the minimum number of subsamples necessary to not exceed the error considered acceptable with respect to the mean of the sample (Alvarez, Steinbach, Bauschen and Enjalbert, 2008). This is one of the operational aspects related to sampling that must be resolved for the inclusion of the technique in the diagnostic protocol.

Sampling Depth

The greatest differences in the measured values of Nan in the same batch and between batches are manifested in the first 5 cm of depth. These differences are not detected if the sampling is done from 0-20 cm.

Cazzoli *et al.,* (2010); Gregoruti *et al.,* (2014); Rodriguez *et al.,* (2015); Garcia *et al.,* (2016) observed for the same batch that from 0-5 cm Nan contents were significantly higher. The differences observed in the Nan value of 0-5 cm in some cases were between 40 and 100% more than that of 5-20 cm.

Gregoruti *et al.,* (2014) explain that the significant differences in the Nan values between depths (0-5 vs 0-20) are due to the variation of the Norg within the macroaggregates, for which a greater expression of the potential can be expected in the field of N mineralization, in relation to that measured through the Nan index, with management practices that tend to break the surface macroaggregates or, as Cazzoli *et al.,* (2010) in environments susceptible to erosive phenomena or in conditions in which these macroaggregates are less stable.

The available bibliography shows that for detailed studies (of quality or health of the soil) in lots under SD, samples of 0-5 and 5 to 20 cm are analysed, while routine studies with diagnostic purposes that seek to evaluate the scope of the measurement of the Nan as predictor of the Nmin use samples of 0-20 cm.

A priori, it is to be expected that the lack of sampling stratification conditions the predictive nature of the Nan, since the measured value can overestimate or underestimate the Nan of the thickness analysed depending on the degree of participation of the first centimeters of depth in the total mass required for measure in such a way that it would be important when defining the sampling section to carry out a stratified sampling.

Characteristics to Consider of the Analytical Technique

A-Soil Mass

Proposed analytical protocol Keeney & Bremner (1966) indicates that 5 g of soil are used to measure the Nan. However, some works such as Genovese *et al.,* (2009) and Orcellet *et al.,* (2016) double the value of the soil mass used to measure the Nan (10 g) while others such as Echeverria *et al.,* (2000) and Gregoruti *et al.,* (2014) carry out the incubation tests with a lower value (3 and 2 g of soil, respectively).

Although the final result is expressed in terms of concentration mg. N kg^{-1} of soil, which makes it independent of the initial mass, changes in the mass used alter the detectability of the technique, which is given by the minimum amount of a substance (defined in terms of absolute quantity) that provides a measurable response by the method used (Garfield, 1991).

B- Incubation Time

Echeverría *et al.,* (2000) and Mariano *et al.,* (2013) carried out anaerobic incubations of soil samples from 0 to 20 cm deep for 7 and 14 days. The authors conclude that given the high and close positive relationship between N_0 and Nan obtained from 7-day incubations, this time would be sufficient to estimate the fraction of N org that corresponds to NPM.

Echeverría *et al.,* (2000) point out that an incubation period of 7 days constitutes a relevant operational advantage when putting this methodology into practice. However, for the routine analysis of a large number of soil samples, this comparative advantage over aerobic incubations becomes the main drawback of the Nan since, as Genovese *et al.,* (2009) the incubation period (minimum 7 days) is relatively long compared to other alternatives in which the measurement of the NPM can be carried out in a few hours (determination of the N-ammonium produced by the treatment of the soil sample with a solution of 2M KCl at 100°C for 4 hours) or in minutes (by steam distillation of the soil sample with a phosphate-borate buffer solution of pH 11.2 for 8 min). Gianello & Bremner, (1986) show that both methods are highly correlated with the results obtained by aerobic and anaerobic (Nan) incubation methods and are considered very suitable for routine use in soil analysis laboratories because, among other operational advantages, they do not require filtration or transfer steps.

This highlights the need for comparative studies to evaluate the predictive capacity of the Nan in relation to other faster analytical techniques.

C-Techniques Used to Measure the N Produced during Anaerobic Incubation

The measurement of the initial ammonium and that produced during the incubation can be carried out by colorimetry, distillation-titration or potentiometry by using a specific electrode.

Pegoraro *et al.,* (2012) compared two methods for determining the initial and produced N. Those methods were colorimetry (COL) and distillation-titration (DEST). The results obtained by both methodologies were similar. The DEST method presented more homogeneous values and with less dispersion, which allowed them to detect significant differences between the treatments evaluated, which is why they suggest the use of the DEST method for the measurement of the Nan because it is more reliable, precise and sensitive than the DEST method Cabbage.

For the inclusion of Nan as a routine technique, it will be important to standardize the mass measurement protocol, incubation time and ammonium reading technique produced all in a single procedure to reduce the variability of the analytical results (Marban and Ratto, 2005).

Interpretation of the Obtained Result

It is important to differentiate NPM from actual or net mineralization (N-min). The difference between both approaches is that the NPM corresponds to the fraction of organic N that can be converted, by the activity of the heterotrophic aerobic microbial biomass, into soluble inorganic forms (NH4+ and NO3-) without environmental restrictions, while the Nmin is the amount of N that is actually mineralized depending on environmental conditions (Martínez *et al.*, 2018).

The analytical data of the Nan measurement correspond to the N produced during a "forced mineralization", in an anaerobic environment and at high temperatures.

Benintende *et al.*, (2007) report a difference of 10% between the NPM measured by long-term aerobic incubations (N_0) in relation to that obtained from the Nan measurement.

So far it has not been possible to fit a meaningful model to estimate Nmin based solely on the Nan measurement (Reussi Calvo *et al.*, 2018).

The result obtained from the Nan measurement can be interpreted as a qualitative indicator of the soil's ability to provide N during the crop cycle.

Final Considerations

The analytical technique used to measure Nan, like most N mineralization tests, has not been calibrated to predict response to nitrogen fertilization. Based on this, although there are operational advantages to include the Nan measurement in the routine protocol since it is a relatively fast and low-cost technique, its use with diagnostic criteria is limited by the uncertainty of its predictive nature of the Nmin added to the lack of information for its application in the soils of the extra Pampas region.

From the technical point of view at the regional level (Córdoba-Argentina), the lack of calibration tests based on long-term aerobic incubation experiments that allow the adjustment of Nan with No and the development of field models that correlate the value of Nan as an indicator of Nmin with crop response to N inputs limits its value as a tool for diagnostic purposes.

From the point of view of the task of the soil laboratories in the area that provide the pre-sowing analysis service for its inclusion in the diagnostic protocol, work should be done on the standardization of the measurement technique so that the results obtained regardless of the laboratory that do it are comparable.

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