



# Nitrogen mineralization indicators under semi-arid and semi-humid conditions: influence on wheat yield and nitrogen uptake

Juan M. Martínez <sup>a</sup>, Juan A. Galantini <sup>b</sup>, Matías E. Duval <sup>c</sup>, María Rosa Landriscini<sup>a</sup>, Ramiro J. García<sup>c</sup>, and Fernando López<sup>c</sup>

<sup>a</sup>Centro de Recursos Naturales Renovables de la Región Semiárida, Universidad Nacional del Sur (UNS)-CONICET, Bahía Blanca, Argentina; <sup>b</sup>Comisión de Investigaciones Científicas (CIC), La Plata, Argentina; <sup>c</sup>Dpto. Agronomía, Universidad Nacional del Sur (UNS), Bahía Blanca, Argentina

## ABSTRACT

The objectives were i) to assess indicators for potential nitrogen (N) mineralization and ii) to analyze their relationships for predicting winter wheat (*Triticum aestivum* L.) growth parameters (yield and N uptake,  $N_{up}$ ) in Mollisols of the semi-arid and semi-humid region of the Argentine Pampas. Thirty-six farmer fields were sampled at 0–20 cm. Several N mineralization indicators, wheat grain yield and  $N_{up}$  at physiological maturity stage were assessed. A principal component (PC) analysis was performed using correlated factors to grain yield and  $N_{up}$ . The cluster analysis showed two main groups: high fertility and low fertility soils. In high fertility soils, combining PCs in multiple regression models enhanced the wheat yield and  $N_{up}$  prediction significantly with a high  $R^2$  (adj  $R^2 = 0.71$ – $0.83$ ). The main factors that explained the wheat parameters were associated with water availability and N mineralization indicator, but they differ according to soil fertility.

**Abbreviations:** N: nitrogen; SOM: soil organic matter; POM: particulate organic matter; SOC: soil organic carbon; SON: soil organic nitrogen; POM-C: particulate organic carbon; POM-N: particulate organic nitrogen;  $N_{an}$ : anaerobic nitrogen;  $N_{hyd}$ : 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;  $N_{up}$ : wheat N uptake;  $NO_3$ -N: inorganic N in the form of nitrate; FR: fallow rainfalls (March-Seeding rainfall); FLR: flowering rainfalls (October–December rainfall); GFR: grain filling rainfall (November rainfall); CCR: crop growing season rainfall (June–December rainfall); PCA: principal component analysis; PC: principal component; MR: multiple regression

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## KEYWORDS

Mollisols; potential N mineralization; principal component analysis; winter crop

## Introduction

Nitrogen (N) is a major yield-limiting nutrient in agricultural areas, especially for grain crops (St. Luce et al. 2011). Understanding N dynamics is crucial for enhancing N-use efficiency and sustainability of production systems (Martínez et al. 2016). It is widely acknowledged that crop response to nitrogen fertilizers is inversely related to the soil's ability for making N readily available to plants (Curtin and MacCallum 2004).

The Argentine Pampean region is known as one of the most important world grain-producing areas, with wheat (*Triticum aestivum* L.), corn (*Zea mays* L.), and soybean (*Glycine max* L. Merr.) as its main crops (Martínez et al. 2017). The wheat crop is the basis of the production systems in a wide

region of the Southwest in Argentina's Pampas (Martínez, Galantini, and Landriscini 2015). Its yields are influenced by weather conditions and soil properties, forcing to maximizing the N use efficiency.

Available soil N is primarily produced by mineralization of soil organic matter (SOM), which supplies 50 to 80% of the N needed by the crop (Kundu and Ladha 1995) and can release N to enhance crop yield in the short term or retain N to maintain crop productivity in the long term. Soil organic N-forms account for as much as 90% of total N in the topsoil. Even though the N-content in soils is high, it is estimated that only 1 to 3% of total organic N is mineralized (Curtin and Wen 1999). Intensive farming practices over the last few years have resulted in decreased SOM content in the Argentine Pampas (Duval et al. 2013). At the same time, conservation systems such as no-tillage (NT) have affected the N-mineralization capacity of soils, due to the increase of the active fraction of organic N as a result of crop residue accumulation on the soil surface (Mikha and Rice 2004). However, the overall effect of this fraction remains unclear in view of studies reporting that organic N enhancement under this system is not necessarily associated with an increase in N-mineralization.

Most estimates on N-supply through soil mineralization are based on long-term aerobic incubations (Martínez and Galantini 2017), but this is a time-consuming method (Walley et al. 2002). Research has focused on developing a number of chemical and biological methods or indicators that can perform estimates in a fast and simple way (Martínez and Galantini 2017). Laboratory procedures often fail to include the environmental factors governing mineralization rates. Chemical methods help determine the pool size of mineralizable N alone, but they do not determine the rate-regulating factors. Biological methods, instead, estimate the pool of mineralizable N and can measure the substrate quality by developing a rate constant.

Some studies have shown that it is possible to explain in part the variability in crop yield by considering soil attributes (Kravchenko and Bullock 2000; Shukla, Lal, and Ebinger 2004), N mineralization indicators (Appel and Mengel 1993; Giroux and Tran 1987) or the combination within soil properties and N mineralization indicators (Nyiraneza et al. 2009). Combining factors could help to explain a greater proportion of the variability in crop yield and  $N_{up}$ . However, as was mentioned by different authors (Bowerman and O'Connell 1990; Martínez, Galantini, and Duval 2018; Nyiraneza et al. 2009) using multiple regressions (MR) could result in multicollinearity between the factors of the model. One way to reduce the multicollinearity and see the relationships of all variables in different dimensions is using 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 (Mallarino, Oyarzabal, and Hinz 1999; Martínez, Galantini, and Duval 2018; Shukla, Lal, and Ebinger 2004).

The N mineralization methodologies can evaluate different mineralized pools of the total soil N (Schomberg et al. 2009; Walley et al. 2002). On the other hand, Kay et al. (2006) reported that estimates on fertilizer needs of crops, which are based on N mineralization indicators, should be combined with weather conditions. As Kravchenko and Bullock (2000) reported, yield variability is caused by several factors; however, the challenge is to identify measurable factors that, in combination, describe an agronomically useful portion of crop variability. Our hypothesis was that wheat yield and  $N_{up}$  can be better predicted by combining N mineralization indicators and rainfalls during the crop growing season, however, this prediction is related to the soil organic fractions (soil fertility). The objectives of this study were i) to assess indicators for potential N mineralization and ii) to analyze their relationships for predicting winter wheat growth parameters (yield and  $N_{up}$ ) in Mollisols of the semi-arid and semi-humid region of the Argentine Pampas. In this study, PCA coupled with MR was used with a set of N mineralization indicators and most important rainfalls that better explain the variations in wheat yield and  $N_{up}$  in soils under these conditions.

## Material and methods

### Study site: soil sampling and crop management

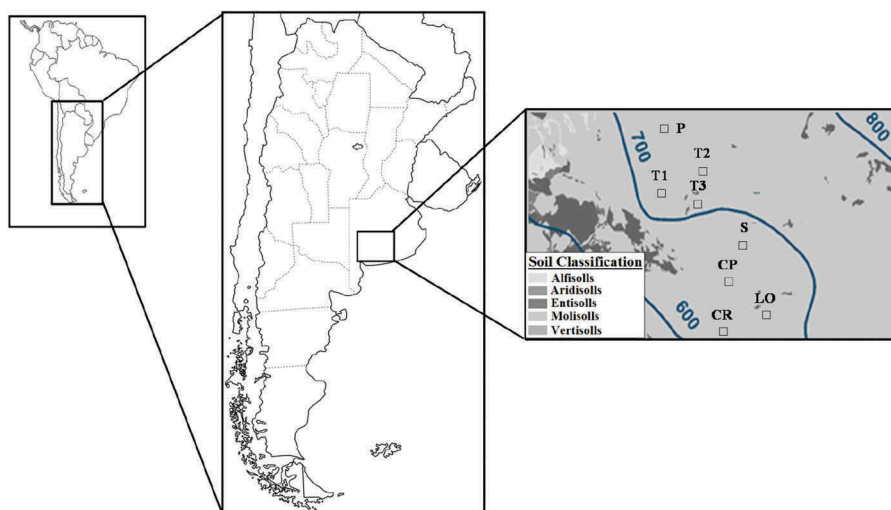
During 2010 and 2011 thirty-six (36) farmer fields cultivated with wheat under NT were sampled. The sites were located in the semi-arid (600–700 mm isohyets) and semi-humid region (700–800 mm isohyets) in the southwest of the Argentine Pampas (Figure 1).

Predominant soils were Mollisols (Typic Argiudoll, Argiudoll and Argiustoll) (Soil Survey Staff 2010) developed in aeolian sediments (loess), with a wide range of depth fluctuation, texture, soil organic carbon content and fertility (Álvarez and Lavado 1998). The rainfall gradient determines an udic soil moisture regime for continental sites and ustic for sites next to the coast. Rainfall amount and frequency are irregular for all sites, the rainiest seasons being in autumn (March–April) and spring (September–October). All soils had been under continuous agriculture for 10–15 years under NT. This system was characterized by the absence of tillage with over 30% residues covering the soil surface in all fields. In general, herbicide (1–2 L ha<sup>-1</sup> of glyphosate) was applied for weed control and for initiating the chemical fallow. When farmers applied fertilizers, fields were fertilized with 10–20 kg P ha<sup>-1</sup> year<sup>-1</sup> as diammonium phosphate (18–46–0) at crop seeding. The wheat seeding was approximately in June–July, whereas, the harvest was at the beginning or mid -December.

Three georeferenced sampling areas of about 50 m<sup>2</sup> were selected in each field ( $n = 36$ ); they were representative of the fields to reduce spatial variability. A composite soil sample (16 and 20 soil cylinders) was collected from each sampling area (replications) at each field. Sampling was performed at 0–20 and 20–60 cm depths in winter, prior to wheat seeding. Site characteristics were shown in Table 1. Data on annual mean, maximum and minimum temperature, annual and crop growing season rainfall were collected from SMN (National Weather Service) weather stations.

### Soil chemical and physical analyses

Soil samples collected at sowing in 0–20 cm depth were air dried and analyzed for the following soil parameters: soil organic carbon (SOC) by dry combustion with a Leco automatic analyzer (Leco Corporation, St Joseph, MI), soil total N (SON) by Kjeldahl method (Bremner 1996), extractable phosphorus (Pe) (Bray and Kurtz 1945) and pH on a 1:2.5 soil-water suspension. In soil samples of



**Figure 1.** Site location in the southwest of the Argentine Pampas. Sites located within the 600–700 mm (CR = Coronel Rosales; LO = Las Oscuras; CP = Coronel Pringles; S = Saldungaray) and 700–800 mm (T1 = Tornquist 1, T2 = Tornquist 2, Tornquist 3, P = Pigüé) isohyet were classified as semi-arid and semi-humid, respectively.

**Table 1.** Soil and climatic characteristics of the sites.

Sites	<i>n</i>	Soil Classification <sup>a</sup>	Previous crop (%)	MAT <sup>b</sup>	Tmax (°C)	Tmin	Rainfall	
							annual (mm)	CCR <sup>c</sup>
Tornquist 1 (T1)	2	Typic Argiudoll	Wheat (50) Sunflower (50)	14	21	8	740	417
Tornquist 2 (T2)	3	Typic Argiudoll	Barley(33) Sunflower (33) Oat (33)	14	21	8	756	388
Tornquist 3 (T3)	2	Typic Argiudoll	Wheat (50) Sunflower (50)	14	21	8	741	497
Pigüé (P)	4	Typic Argiudoll	Soybean (100)	14	20	7	800	464
Coronel Rosales (CR)	3	Entic Haplustoll	Barley (100)	15	21	9	664	285
Las Oscuras (LO)	12	Typic Argiustoll	Wheat (50); Pea (25);Maize (25)	15	21	9	669	317
Coronel Pringles (CP)	2	Typic Haplustoll	Wheat (50); Barley (50)	15	21	8	686	331
Saldungaray (S)	8	Typic Argiustoll	Wheat (64); Sunflower(24); Virgin (12)	15	21	8	694	307

<sup>a</sup>Soil Survey Staff (2010). <sup>b</sup>MAT, mean annual temperature. <sup>c</sup>Crop growing season rainfall. Tmax, annual mean maximum temperature; Tmin, annual mean minimum temperature. *n*, number of fields per site. Previous crop, in parenthesis % of the previous crop in the *n* fields per site. Predecessor crops: Wheat (*Triticum aestivum* L.); Barley (*Hordeum vulgare* L.); Sunflower (*Helianthus annuus* L.); Oat (*Avena sativa* L.); Soybean (*Glycine max.* L Merr.); Pea (*Pisum sativum* L.).

0–20 and 20–60 cm, available N as form of total inorganic N (NO<sub>3</sub>-N+NH<sub>4</sub>-N) was analyzed by steam distillation (Mulvaney 1996).

Soil texture was estimated by particle size fractionation of SOM (Duval et al. 2013), subtracting the percentage of SOC from each fraction (higher and lower than 53 microns) and estimating the sand and the fraction silt plus clay. The soil chemical and physical properties are shown in Table 2.

### Crop yield and crop N-uptake

At physiological maturity, the above-ground biomass of wheat was harvested manually from two 0.25 m<sup>2</sup> areas per sampling point. The dry matter was determined after drying in a forced-air oven at 60°C for at least 72 hours. The grain was separated from the straw and they were both weighed. The N concentration in the total aerial biomass (grain and straw) was determined by the standard micro-

**Table 2.** Soil properties at 0–20 cm and nitrogen availability at 0–60 cm of the sites.

Sites	<i>n</i>	SOC	Available N	Pe	pH	silt+clay
		(g kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )			(g kg <sup>-1</sup> )
T1	2	19.2(1.5)	8.1(4.0)	6(1.2)	6.3(0.2)	480(89)
T2	3	20.0(5.5)	7.4(7.6)	28(16)	6.4(0.1)	567(86)
T3	2	21.8(1.9)	6.7(7.4)	26(2.8)	6.4(0.4)	606(61)
P	4	14.3(5.0)	30(14)	15(5.4)	6.2(0.2)	411(171)
CR	3	14.4(1.5)	13 (13)	3(2.9)	6.1(0.2)	536(101)
LO	12	10.0(1.9)	11(7.7)	12(0.5)	6.0(0.2)	344(150)
CP	2	26.3(4.8)	7.9(4.2)	13(7.1)	6.5(0.8)	618(82)
S	8	21.3(7.2)	27(21)	10(8.5)	7.1(0.6)	523(67)

*n*, number of fields by site; SOC, soil organic carbon (g kg<sup>-1</sup>); Available N, soil inorganic nitrogen (NO<sub>3</sub>-N+NH<sub>4</sub>-N) at crop seeding at 0–60 cm (mg kg<sup>-1</sup>); Pe, extractable phosphorus (mg kg<sup>-1</sup>). Values in brackets indicate standard deviation.

Kjeldahl method (Bremner 1996); the values obtained were then used to estimate  $N_{up}$  by the aerial biomass of the wheat ( $\text{kg N ha}^{-1}$ ). Grain yield and  $N_{up}$  were considered as wheat parameters across the manuscript.

### **Indicators of nitrogen mineralization**

Several methods were evaluated to analyze the potential N-mineralization at 0–20 cm soil layer: short-term anaerobic incubation ( $N_{an}$ ) (Waring and Bremner 1964); hot chemical hydrolyzable N ( $N_{hyd}$ ) (Gianello and Bremner 1986);  $N_{205}$  and  $N_{260}$  (MacLean 1964); SON (Schomberg et al. 2009); N in particulate organic matter (POM-N) (Sharifi et al. 2007), and  $\text{NO}_3\text{-N}$  (Spargo et al. 2009). Data are presented in concentrations ( $\text{g kg}^{-1}$ ;  $\text{mg kg}^{-1}$ ).

### **Anaerobic nitrogen**

Anaerobic nitrogen ( $N_{an}$ ) was determined following the method by Waring and Bremner (1964) in a short-term anaerobic incubation. Briefly, 5 g of soil was put into a test tube and 25 mL of distilled water was added. The tube caps were securely tightened and then incubated at 40°C for 7 days under anoxic conditions. After incubation, the samples were transferred to a distillation flask and 25 mL of 4 mol  $\text{L}^{-1}$  potassium chloride (KCl) was added; ammonium ( $\text{NH}_4$ )-N was determined by steam-distillation (Mulvaney 1996). The  $N_{an}$  was calculated by subtracting the quantity of inorganic N -extracted with 2 mol  $\text{L}^{-1}$  KCl in non-incubated samples at room temperature- from the amount in the incubated extract.

### **Chemical hydrolyzable nitrogen**

Labile N was chemically extracted by soil digestion with a strong salt solution of 2 mol  $\text{L}^{-1}$  KCl, as described by Gianello and Bremner (1986). The procedure consisted in digesting 3.00 g of soil in 20 mL of the solution at 100°C for 4 hours in a block digester. Then, the sample was 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 temperature and hydrolyzable N ( $N_{hyd}$ ) was determined.

### **Cold nitrate-extraction**

Briefly, 5.00 g of air-dried soil was added to 50 mL of 2 mol  $\text{L}^{-1}$  KCl. Samples were shaken for 30 min at 290 rpm and centrifuged. The extract was filtered and N in nitrate form ( $\text{NO}_3\text{-N}$ ) was determined by steam distillation (Mulvaney 1996).

### **Determination by UV-visible spectrophotometry**

The procedure was performed according to the method described by MacLean (1964). Five grams of soil were added to 100 mL of 0.01 mol  $\text{L}^{-1}$   $\text{NaHCO}_3$ . The suspension was shaken for 15 min in a 250mL Erlenmeyer flask. The samples were centrifuged and the suspensions were filtered through a Whatman 42 filter paper. Then, absorbance was measured at different wavelengths (200, 205, 210, 220, 230, 240, 250, 255, 260 and 270 nm). However, this study reported data for 205 ( $N_{205}$ ) and 260 nm ( $N_{260}$ ) wavelengths. When making measurements at 200, 205 and 210 nm, two drops of concentrated HCl were added to remove carbonate wavelength peaks, as these ranges may be absorbed. It is important to note that carbonates were not found in any of the samples. Quartz cells and a T60U UV-visible spectrophotometer (PG Instruments) were used for measuring.

### **Soil physical fractionation by particle size**

Fractionation of SOM by particle size was carried out by wet sieving (Duval et al. 2013). Briefly, 50 g of previously air-dried soil was sieved (2 mm) and dispersed in 120 mL glass vials with 100 mL of distilled water. Ten glass beads (5 mm in diameter) were added to increase aggregate fragmentation and reduce potential problems created by different sand contents. The samples were mechanically

dispersed in a rotary shaker for about 16 hours (overnight) at 40 rpm to disintegrate the aggregates. They were then passed through a pair of 53 and 100 micron diameter mesh sieves, which were moved back and forth until the water passing through the finest mesh sieve was clear to the naked eye. Three different fractions were thus obtained: i) a coarse fraction (100–2000 microns) containing coarse particulate organic carbon (cPOM-C) and medium plus coarse sands; ii) a medium fraction (53–100 microns) with the fine particulate organic carbon (fPOM-C) and very fine sand; iii) a fine fraction (<53 microns) which included the mineral-associated organic carbon and silt plus clay minerals. The fine fraction was not used in this study. Carbon content in the coarse and medium fractions was determined using the same method as for SOC determination. Total particulate organic carbon (POM-C) was obtained by adding cPOM-C and fPOM-C contents. The N in the cPOM-N and fPOM-N (cPOM-N + fPOM-N = POM-N) was determined using the same method as for SON.

### **Statistical analysis**

The cluster analysis was performed for grouping soil according to soil organic fractions (SOC, SON, POM-C; POM-N), using Ward's minimum variance method (Ward 1963). The cluster cutting was performed in 50% of the total distance (Balzarini et al. 2008). Differences between N mineralization indicators by Cluster were analyzed using ANOVA. Descriptive statistics and differences of grain yield and  $N_{up}$  were performed by groups of soil by ANOVA with least square difference at 0.05. The N mineralization indicators were analyzed with descriptive statistics. Pearson's correlation analysis was performed among grain yield and  $N_{up}$  with N mineralization indicators and rainfalls: fallow rainfalls (FR) (Mar-Jul), Flowering rainfall (FLR) (Sept-Oct), grain filling rainfall (GFR) (November) and crop growing season rainfall (CCR) (Jul-Nov). The PCA was used to group the highly correlated variables in terms of a few factors. It was performed on the significant correlated N mineralization indicators and rainfalls to the wheat parameters. This multivariate 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; Martínez, Galantini, and Duval 2018). 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 PC>1 by multivariate analysis for predicting grain yield and  $N_{up}$  (dependent variables) by means of 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 maximize the prediction of them. All statistical analyses were performed with Infostat software (Di Rienzo et al. 2013).

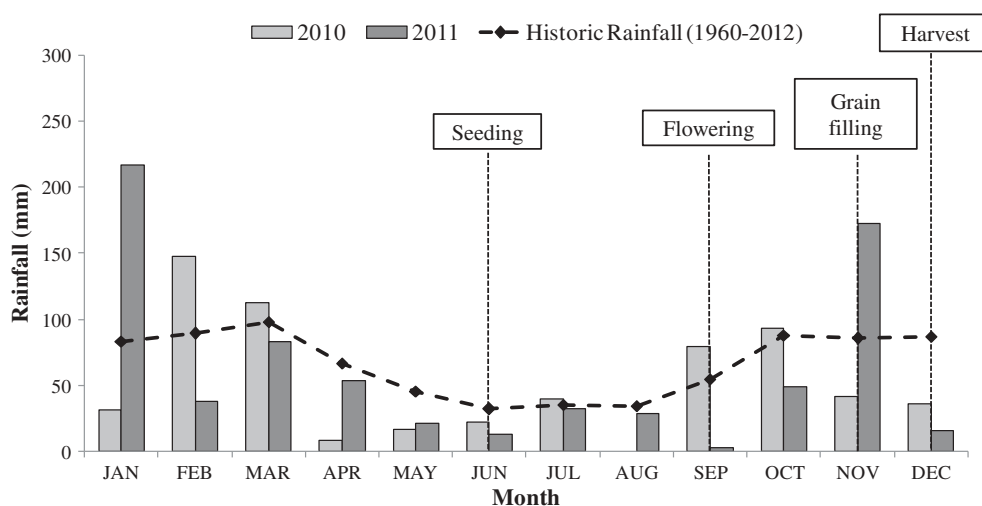
## **Results and discussion**

### **Rainfall conditions**

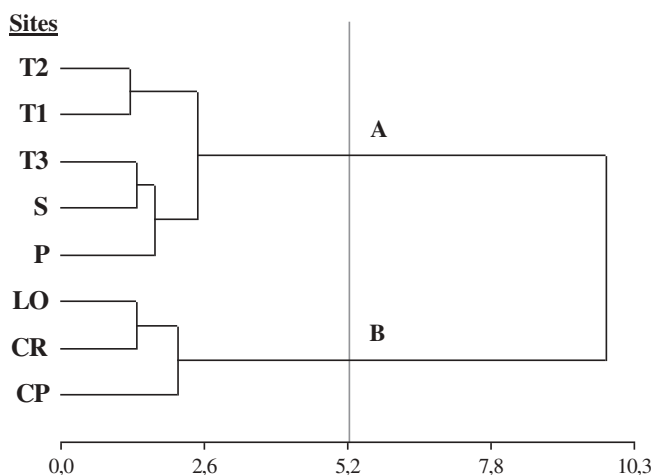
In general, rainfall was concentrated in the periods from January to March and September to December, with variations between years (Figure 2). In 2010, rainfall was marked by periods of drought with respect to the historical rainfall, during the winter months and the grain filling period. In 2011, the drought was in September and October (spring), increasing significantly in November, coinciding with the grain filling period of the wheat. Accumulated rainfalls by year were: 693 and 777 for 2010 and 2011, respectively.

### **Grouping soils by fertility**

The cluster analysis based on soil organic C and N fractions showed two main clusters (Figure 3). Cluster A contained five sites ( $n = 19$ ) with high proximity, and Cluster B included three sites



**Figure 2.** Monthly rainfall (average) of all sites for 2010, 2011 and historic rainfall (1960–2012).



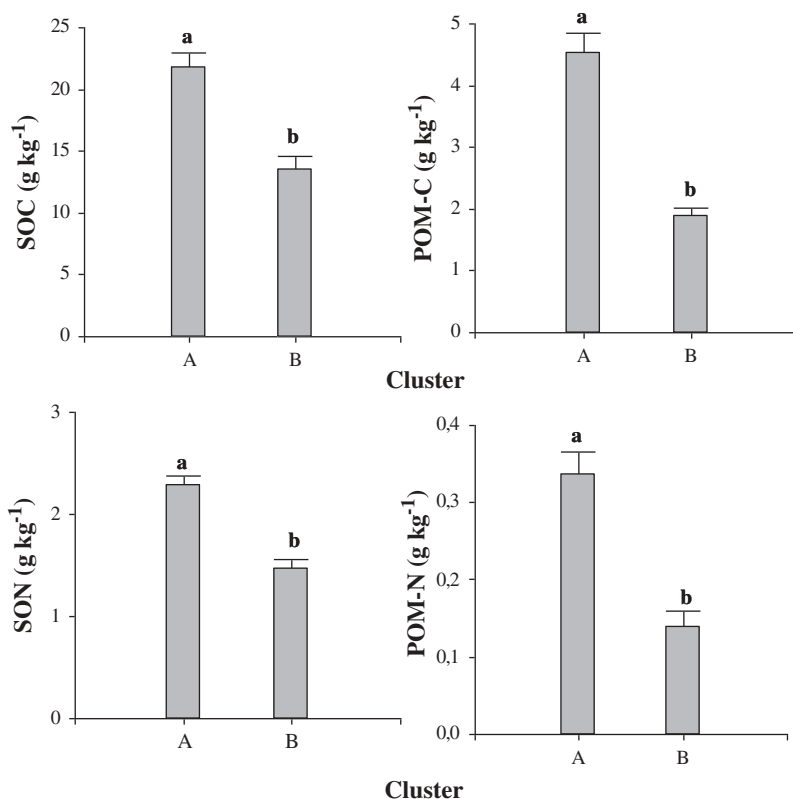
**Figure 3.** Dendrogram of the similarities among soil organic fractions using Ward's minimum variance method.

( $n = 17$ ). The differences between edaphic properties considered for cluster analysis are detailed in [Figure 4](#). Significant differences ( $P < 0.05$ ) in SOC; POM-C; silt and clay, SON and POM-N were found by grouping cluster, with higher values in Cluster A. This showed that soils could be grouped according to their fertility: Cluster A, high fertility soils and Cluster B, low fertility soils. Because many of the methods used to estimate N availability measure, in part, the release of N from some component of the SOM pool (Walley et al. 2002), it is important to analyze the differences in SOM and its fractions when analyzing the N mineralization indicators in different soils. The cluster analysis allows separating soils in accordance with their fertility.

### **Wheat yield and N uptake at physiological maturity**

Significant differences in grain yield and  $N_{up}$  were found between groups of soil. Wheat yields were 2823 and 2121 kg ha<sup>-1</sup> for high fertility and low fertility soils, respectively ([Figure 5](#)). Considering





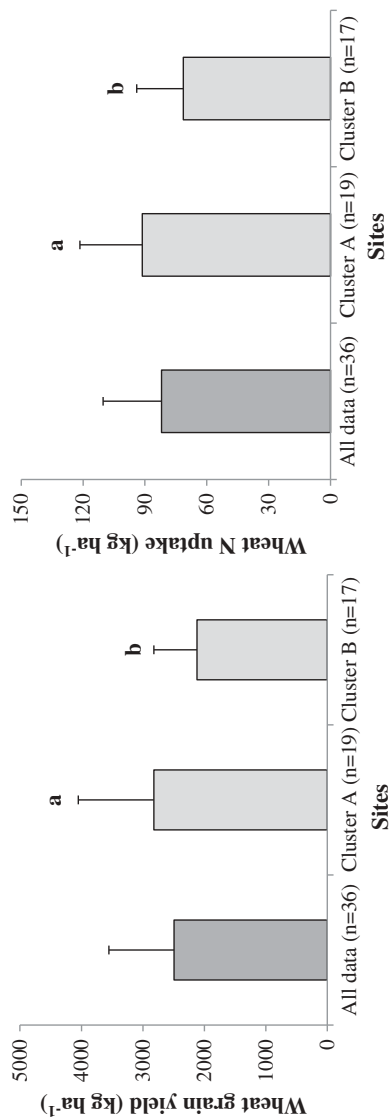
**Figure 4.** Mean values of soil properties evaluated for cluster analysis. Cluster A = high fertility soils; Cluster B = low fertility soils. Vertical bars indicate the standard error. Different letters indicate significant differences between the soil organic matter fractions ( $P < 0.05$ ).

the grain yield, a lower coefficient of variation (CV) was observed in low fertility soils, showing a better stability in yields across sites (data not shown). The  $N_{up}$  were 91.3 and 71.4  $\text{kg ha}^{-1}$  for high fertility and low fertility soils, respectively (Figure 5). For this variable, no differences were obtained in the CV regarding soil fertility (data not shown). It is important to note that separating soils according to soil fertility allowed finding differences in grain yield and  $N_{up}$ .

### Nitrogen mineralization indicators

The results of the N mineralization indicators are presented in Table 3. Broad ranges in values of N mineralization indicators were observed considering all soils, thus showing a greater coefficient of variation ( $CV = 35\text{--}80\%$ ) (data not shown). The ranges for each wavelength ( $N_{205}$  y  $N_{260}$ ) are similar to those reported by Hong, Fox, and Piekielek (1990) in 49 N-fertilized maize (*Zea mays* L.) assays in Pennsylvania, and by Sharifi et al. (2007) in the top 15 cm of soils under different crops and in different climates. Serna and Pomares (1992) observed that the highest values of  $N_{205}$  and  $N_{260}$  were found in sandy soils and the lowest values in clay soils; however, in this study no differences were observed because the soils in the studied area are coarse-textured. The average  $N_{an}$  values varied within a range also cited by other authors (Sahrawat 1983; Schomberg et al. 2009), who worked on soils with different edaphic conditions. The lower standard deviation in  $N_{hyd}$ , confirmed the lower variation in values as reported by Wang et al. (2001), who worked on different soils in Eastern





**Figure 5.** Grain yield, crop N uptake for all data, soil groups and analysis of the variance between soil groups. Vertical bars indicate standard deviation. Different letter indicate significant differences on wheat parameters between soil groups ( $P < 0.05$ ).

**Table 3.** Indicators of nitrogen mineralization by site and soil group.

Sites	Fields per site	N <sub>205</sub>	N <sub>260</sub>	SON	POM-N	N <sub>an</sub>	N <sub>hyd</sub>	NO <sub>3</sub> -N
		(Absorbance)		(g kg <sup>-1</sup> )			(mg kg <sup>-1</sup> )	
T1	2	1.37(0.04)	0.43(0.06)	1.96(0.01)	0.27(0.05)	52.1(33)	22.9(4.9)	8.5(9.5)
T2	3	1.80(0.26)	0.43(0.11)	1.7(0.55)	0.21(0.11)	55.1(19)	28.8(4.6)	9.3(7.4)
T3	2	2.62(0.80)	0.42(0.15)	1.8(0.12)	0.33(0.04)	50.6(3.1)	35.0(1.3)	15(5.9)
LO	12	1.55(0.60)	0.57(0.23)	1.29(0.32)	0.13(0.09)	29.7(10)	20.3(6.1)	3.9(1.2)
CP	2	1.07(0.33)	0.38(0.15)	1.14(0.32)	0.15(0.14)	36.5(8.1)	23.5(2.9)	20.1(15.4)
CR	3	1.61(0.56)	0.49(0.25)	1.02(0.22)	0.17(0.04)	14.6(4.2)	25.8(6.8)	5.1(4.7)
P	4	1.81(0.28)	0.87(0.52)	2.23(0.34)	0.36(0.07)	71.3(9.4)	35.9(8.7)	4.3(5.2)
S	8	1.58(0.63)	0.44(0.14)	1.92(0.39)	0.38(0.19)	47.7(22)	21.4(5.4)	6.0(7.4)
Cluster A	19	1.75(0.57)	0.52(0.30)	1.94(0.38)	0.33(0.14)	54.6(20)	27.2(8.3)	11.1(12.7)
Cluster B	17	1.50(0.57)	0.53(0.22)	1.22(0.31)	0.14(0.08)	27.9(11)	21.6(6.0)	13.2(6.7)
<b>P-value</b>		0.2091	0.9126	<b>0.0001</b>	<b>0.0001</b>	<b>0.0001</b>	<b>0.0001</b>	<b>0.0289</b>

Bold letters indicate significant differences ( $P < 0.05$ ) in N mineralization indicators between groups of soil. N<sub>205</sub>, N determined by spectrometer at 205 nm (absorbance); N<sub>260</sub>, N determined by spectrometer at 260 nm (absorbance); N<sub>an</sub>, anaerobic N (mg kg<sup>-1</sup>); N<sub>hyd</sub>, N hydrolyzed by chemical digestion (mg kg<sup>-1</sup>), SON, soil total N (g kg<sup>-1</sup>); POM-N, N in particulate soil organic matter (mg kg<sup>-1</sup>); NO<sub>3</sub>-N, inorganic N in the form of nitrate (mg kg<sup>-1</sup>). Values in brackets indicate standard deviation.

Australia. However, these values were higher than those reported by Schomberg et al. (2009), who carried out their research in the southeast of USA at 0–5 and 5–15 cm depths.

Considering groups of soil, significant differences between them were found in SON, POM-N, N<sub>an</sub> and N<sub>hyd</sub>, with greater values in high fertility soils (Table 3). These differences between N mineralization indicators evidenced that these indicators were different in accordance with soil fertility.

Considering the correlations among N mineralization indicators (data not shown), highly significant relationships ( $P < 0.01$ ) were found between N<sub>an</sub> and SON ( $r = 0.69$ ), POM-N and SON ( $r = 0.70$ ); POM-N and N<sub>an</sub> ( $r = 0.45$ ); N<sub>hyd</sub> and N<sub>205</sub> ( $r = 0.41$ ); N<sub>hyd</sub> and N<sub>an</sub> ( $r = 0.43$ ). Analyzing the indicators that showed significant differences between soil groups, in high fertility soils highly a significant correlation ( $P < 0.05$ ) was found between POM and SON ( $r = 0.53$ ), whereas a significant correlations between N<sub>an</sub> and SON ( $r = 0.64$ ) was found in low fertility soils.

### Prediction of crop yield and nitrogen uptake at physiological maturity

#### Relationships between grain yield and crop N uptake with mineralization indicators

Analyzing the relationships between grain yield and N<sub>up</sub> with the N mineralization indicators and considering all data ( $n = 36$ ), scarce and significant relationships ( $P < 0.05$ ) were found ( $r = 0.36$ – $0.66$ ) (Table 4). The lack of high correlations between N mineralization indicators and grain yield or N<sub>up</sub> suggests that N-cycling processes and crop productivity may be controlled by different sets of factors in these soils (Turner et al. 1997). Regarding soil groups, in high fertility soils highly significant and positive correlations were observed in grain yield and N<sub>up</sub> with N<sub>260</sub> and N<sub>hyd</sub> ( $r \geq 0.73$ ); whereas, no significant correlation was found in both crop variables with the N

**Table 4.** Pearson's correlations between crop variables and N mineralization indicators.

		Nitrogen mineralization indicator						
		N <sub>205</sub>	N <sub>260</sub>	SON	N <sub>an</sub>	N <sub>hyd</sub>	POM-N	NO <sub>3</sub> -N
.	Soils/group of soil	coefficient of correlation (r)						
Grain yield	All data	0.14	<b>0.45</b>	<b>0.36</b>	<b>0.53</b>	<b>0.66</b>	0.19	<b>−0.48</b>
	A	0.16	<b>0.73</b>	0.28	0.51	<b>0.78</b>	−0.01	−0.55
	B	−0.10	−0.15	−0.07	0.19	0.18	−0.13	−0.18
N uptake	All data	0.23	<b>0.41</b>	<b>0.38</b>	<b>0.40</b>	<b>0.65</b>	0.27	−0.30
	A	0.31	<b>0.75</b>	0.37	0.33	<b>0.75</b>	0.18	−0.30
	B	−0.03	−0.16	−0.16	−0.01	0.28	−0.23	−0.25

In bold letter correlations significant at  $P < 0.05$ . See abbreviations in Table 4.

mineralization indicators in low fertility soils. Similar results were obtained by Walley et al. (2002), who reported high relationships between  $N_{hyd}$  and wheat yield in the comparison of different mineralization indicators under NT in semi-arid regions of Canada.

### *Relationships between grain yield and crop N uptake with crop growing season rainfalls*

Considering all data, significant ( $P < 0.05$ ) and positive correlations were found between grain yield and  $N_{up}$  with FR; FLR; GFR and CCR (Table 5). Regarding soils groups, in high fertility soils were found the same correlations in grain yield as considering all data, whereas, in low fertility soils only significant correlations were found between grain yield with FR and FLR.

### *Grouping N-mineralization indicators in principal components*

Regarding the significant correlated variables (mineralization indicators and rainfalls) with grain yield, the PCA showed eigenvalues  $>1$  for the first three PCs, which accounted for 81% of the variance (Table 6). The results showed that factors that explained grain yield accumulation were associated with water availability and to N mineralization indicators ( $NO_3-N$ ;  $N_{260}$ ). The first PC explained 53% of the total variance and had high positive loadings for FR, FLR and GFR. The second PC explained 15% of the variance and had positive loading for  $NO_3-N$ . The third PC explained 13% of the variance and had high and positive loading for  $N_{260}$ . Considering the significant correlated variables (mineralization indicators and rainfalls) with  $N_{up}$ , the PCA retained only the two first PCs  $>1$  accounting for 72% (Table 6). In this case, the factors that explained the variance were related

**Table 5.** Pearson's correlations between crop variables and rainfalls during growing season.

		Rainfalls			
		FR	FLR	GFR	CCR
Crop variable	Soil/Groups of soil	coefficient of correlation (r)			
Grain yield	All data	<b>0,66</b>	<b>0,68</b>	<b>0,53</b>	<b>0,63</b>
	A	<b>0,62</b>	<b>0,65</b>	<b>0,55</b>	<b>0,71</b>
	B	<b>0,61</b>	<b>0,58</b>	−0,01	0,48
N uptake	All data	<b>0,51</b>	<b>0,58</b>	<b>0,37</b>	<b>0,62</b>
	A	0,46	0,45	0,33	<b>0,61</b>
	B	<b>0,57</b>	<b>0,61</b>	−0,03	<b>0,53</b>

In bold letter correlations significant at  $P < 0.05$ . FR, fallow rainfalls (March-Seeding rainfall); FLR, flowering rainfalls (October-December rainfall); GFR, grainfilling rainfall (November rainfall); CCR, crop growing season (June-December rainfall).

**Table 6.** Results of principal component analysis (PCA) for correlated variables with grain yield and crop N uptake.

	Grain yield ( $PC_{gy}$ )			N uptake ( $PC_{Nup}$ )	
	PC1	PC2	PC3	PC1	PC2
Eigenvalue	4.81	1.31	1.14	4.62	1.15
Proportion of variance	0.53	0.15	0.13	0.58	0.14
Total variance	0.53	0.68	0.81	0.58	0.72
Variable	Eigenvectors				
$N_{260}$	0.11	-0.12	<b>0.81</b>	0.12	<b>0.83</b>
SON	0.30	0.59	0.04	0.33	-0.07
$N_{an}$	0.35	0.34	0.08	0.36	0.01
$N_{hyd}$	0.28	-0.12	0.46	0.28	0.48
$NO_3-N$	-0.22	<b>0.70</b>	0.09	-	-
FR	<b>0.41</b>	-0.12	-0.17	<b>0.42</b>	0.14
FLR	<b>0.43</b>	-0.09	-0.16	<b>0.43</b>	-0.13
GFR	<b>0.39</b>	-0.004	-0.23	<b>0.39</b>	-0.23
CCR	0.38	-0.02	-0.02	0.38	-0.01

$N_{260}$ , N determined by spectrometer at 260 nm;  $N_{an}$ , anaerobic N;  $N_{hyd}$ , N hydrolyzed by chemical digestion, SON, soil total N;  $NO_3-N$ , inorganic N in the form of nitrate; FR, fallow rainfall; FLR, flowering rainfall; GFR, grain filling rainfall, CCR, crop growing season rainfall.

to water availability and with a N mineralization indicator ( $N_{260}$ ), being similar to the grain yield. The first PC explained 58% of the total variance and had high positive loadings for FR, FLR and GFR. The second PC explained 14% of the variance and had positive loading for  $N_{260}$ . These results indicated that rainfalls were the main factors affecting grain yield or  $N_{up}$ , because they explained the majority of the variance in both wheat parameters (Table 6).

### Prediction of yield and crop N uptake using multivariate analysis

Considering all data, combining PCs in MR models enhanced the wheat yield prediction (Table 7) with a moderate coefficient of determination ( $\text{adj } R^2 = 0.63$ ;  $n = 36$ ), however, the  $N_{up}$  prediction was not substantially improved ( $\text{adj } R^2 = 0.50$ ). From the PCs generated from correlated variables to grain yield and  $N_{up}$  (Table 6), the stepwise model selected 2 and 2 PCs for grain yield and  $N_{up}$ , respectively (Table 7).

Analyzing the data according to soil fertility, in high fertility soils the prediction of grain yield and  $N_{up}$  was improved significantly with a high  $R^2$  for both parameters ( $\text{adj } R^2 = 0.71$ – $0.83$ ), whereas in low fertility soils a scarce prediction was detected for both wheat parameters (Table 7). These results confirmed the hypothesis that the prediction of wheat parameters (grain yield and  $N_{up}$ ) depends on the soil fertility. In this study, it would be verified that in the most fertile soils (higher SOC content and organic fractions) the N mineralization indicators could partially explain the wheat yield variability. Also, it could be observed that in high fertility soils, the PC that explain the variability in grain yield and  $N_{up}$  included the rainfalls (FR, FLR, GFR) and a N mineralization indicator ( $N_{260}$ ), whereas, in low fertility soils the main variable that affect the wheat parameters was the PC highly influenced by the rainfalls (FR, FLR, GFR) (Table 6). This could confirm that water factor is more important than N in low fertility soils. This result could be attributed not only to the positive effect of SOC on water retention (Díaz-Zorita, Buschiazzo, and Peinemann 1999) but also as a probable result of a high N mineralization with a high SOM content (Alvarez and Grigera 2005; Álvarez, Álvarez, and Steinbach 2002). In addition, Walley et al. (2002) suggest that in semi-arid regions water remains as the key factor that controls the demand and availability of N. It is important to note that low fertility soils were located in the semi-arid region of the Argentine Pampas. Moreover, due to the variability in the rainfall frequency and the water deficit occurred during the crop growing season (Figure 2), the prediction of wheat parameters by rainfall was scarce in these soils, because the erratic rainfall in

**Table 7.** Prediction of grain yield and crop N uptake through multiple regressions.

Dependent variable	Soils/Group of soils	<i>n</i>	Regressor variable	Estimated parameter	Standard error	P-value	$R^2$	Adj $R^2$
Grain yield	All data	36	Intercept	2492	107.5	<0.0001	0.65	0.63
			PC <sub>gy</sub> 1	361.8	49.7	<0.0001		
			PC <sub>gy</sub> 2	304.4	102.3	0.0054		
	Cluster A		Intercept	2256	143.0	<0.0001	0.85	0.83
			PC <sub>gy</sub> 1	430.9	56.8	<0.0001		
			PC <sub>gy</sub> 3	466.3	97.8	0.0002		
	Cluster B		Intercept	3374	365.6	<0.0001	0.47	0.44
			PC <sub>gy</sub> 1	793.4	216.9	0.0023		
N uptake	All data	36	Intercept	81.9	3.4	<0.0001	0.53	0.50
			PC <sub>Nup</sub> 1	8.3	1.6	<0.0001		
			PC <sub>Nup</sub> 2	9.7	3.2	0.0045		
	Cluster A		Intercept	82.5	4.7	<0.0001	0.74	0.71
			PC <sub>Nup</sub> 1	7.7	1.9	<0.0001		
			PC <sub>Nup</sub> 2	15.0	3.1	0.001		
	Cluster B		Intercept	111.7	13.2	<0.0001	0.47	0.41
			PC <sub>Nup</sub> 1	25.1	7.8	0.0055		

All prediction equations are constructed in the form  $y = \beta_0 + x_1\beta_1 + x_2\beta_2$ , where  $\beta_0$  is the intercept,  $\beta_1$ ,  $\beta_2$  are the parameter estimates and  $x_1$ ,  $x_2$  are the new variables generated by PCA.

these regions, does not often allow high yields (Martínez, Galantini, and Landriscini 2015; Martínez et al. 2016).

These results may indicate that the relationships between wheat parameters and N mineralization indicators could be due to fertility conditions, which in turn are highly influenced by the weather conditions. For that reason, climatic variables should be used in these models to confirm these relationships –especially in semi-arid and semi-humid regions–, in view of their strong influence on crop yield and N-uptake. In addition to these results, Kay et al. (2006) reported that estimates on fertilizer needs of crops, which are based on mineralization indicators, should be combined with weather conditions. This is because the climate affects not only soil N-dynamics but also crop response to N-fertilizer after the indicator had been measured.

Results obtained showed that grouping soils according to their fertility by the cluster analysis, probably reduces the variability in the soil resources and increases the prediction of wheat parameters. Other studies (Robertson et al. 1997; Walley et al. 2002) had shown that with a high degree of variability for all soil resources, it should not be expected a high degree of correlation between any single measure of N availability and either crop growth or  $N_{up}$ . This study would allow a better understanding about what is happening with N pools and the plant-soil systems in sites with low water availability during the wheat growing season.

## Conclusion

The main factors that explained the wheat grain yield and N accumulation were associated with water availability and N mineralization indicators, but these relevant factors were different considering soil fertility.

Use of N mineralization indicators alone failed to predict wheat parameters accurately independently of soil fertility in these Mollisols of the semi-arid and semi-humid region. Instead, grain yield and  $N_{up}$  by wheat were predicted with a good degree of adjustment by using MR combining PCs in soils with high fertility. On the other hand, the  $N_{260}$  seems to be a promising indicator for evaluating grain yield and N accumulation in high fertility soils. Also, the simplicity, speed, and cost effectiveness of this indicator suggests that it may use as a routine soil N mineralization under these soil conditions. Therefore, the usefulness of these N mineralization indicators to make predictions for N mineralization and wheat growth on less fertility soils may be limited, because the water factor was the main factor. For this reason, it is important to group sites according to soil fertility.

Additional work is needed to relate the potential N mineralization indicator to the N availability for the wheat response to allow appropriate adjustments in N fertilizer rates and management in this environment with low water availability.

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## ORCID

Juan M. Martínez  <http://orcid.org/0000-0002-7198-8066>

Juan A. Galantini  <http://orcid.org/0000-0002-4536-8605>

Matías E. Duval  <http://orcid.org/0000-0002-8731-9107>

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