Anaerobically Incubated Nitrogen Improved Nitrogen Diagnosis in Corn

Juan Orcellet,* Nahuel Ignacio Reussi Calvo, Hernán Rene Sainz Rozas,
Nicolás Wyngaard, and Hernán E. Echeverría

ABSTRACT

Current N diagnostic methods for corn (*Zea mays* L.) are often based on the nitrate nitrogen (NO_3^-–N) concentration before planting (pre-plant nitrate test, PPNT) and nitrate nitrogen (NO_3^-–N) concentration at V6 stage (PSNT). These tests provide scant information on soil N mineralization during the growing season, which can supply a considerable proportion of corn N requirements. The objective of our study was to evaluate if in-season N recommendations could be improved by inclusion of a N mineralization potential estimator. We conducted field experiments ($n = 35$) in three different areas and in two planting dates. At each site we evaluated PPNT, PSNT, and NH_4–N released during anaerobic incubation (N_{an}), which were then related to corn yield in unfertilized plots (0N) and corn response to nitrogen fertilization ($N_{resp\%}$) using multiple regression analysis. The sole incorporation of N_{an} to PPNT and PSNT models improved their capacity to predict corn yield in 0N plots and $N_{resp\%}$ only in areas with similar edaphic-climatic characteristics. Independently of the geographical region, when PPNT and PSNT were combined with N_{an}, texture, and temperature, their capacity to predict yield in 0N plots was increased (PPNT: from R^2 0.02–0.47; PSNT: from R^2 0.09–0.53), as it was their capacity to estimate $N_{resp\%}$ (PPNT: from R^2 0.06–0.23; PSNT: from R^2 0.19–0.42). The inclusion of N_{an} can improve traditional N diagnostic models when it is combined with edaphic/climatic properties that account for the mineralization rate of this N pool.

Core Ideas

- Traditional corn N diagnostic methods (pre-plant nitrate N test and pre-sidedress nitrate N test) only account for mineral N.
- Objective: to improve N diagnostic methods by considering N mineralization.
- Pre-plant nitrate N test and pre-sidedress nitrate N test were improved by anaerobic-N (N_{an}) in areas with similar soil/climates.
- Models combining N_{an}, texture and temperature improved pre-plant nitrate N test and pre-sidedress nitrate N test in all areas.

Published in Agron. J. 109:1–8 (2017)
doi:10.2134/agronj2016.02.0115
Received 16 May 2016
Accepted 23 Sept. 2016

NITROGEN FERTILIZER RECOMMENDATIONS for corn are often based on the quantification of soil NO_3–N content before sowing at a 0- to 60-cm depth (PPNT) (Magdoff et al., 1984; Bundy and Meisinger, 1994; Sainz Rozas et al., 2008). To determine the N fertilizer rate, the measured PPNT value is compared to availability thresholds which vary depending on the area, tillage system, and yield goal (Echeverría et al., 2014). However, Brouder and Mengel (2003) demonstrated that PPNT failed to predict corn response to N fertilization in 39% of the evaluated cases (301 field experiments). This is because PPNT only accounts for a small fraction of the total available N, which is gradually released by the mineralization of soil organic matter (SOM) throughout the growing season. For example, a 22 mg kg^{-1} PPNT critical level (Brouder and Mengel, 2003) represents only 9% of the final N corn uptake (Bender et al., 2013).

Another approach commonly used to calculate the N fertilizer requirement for corn is based on measuring the NO_3^--N concentration in the top 30 cm of the soil at the V_6 (Ritchie and Hanway, 1982) corn stage (pre-sidedress soil nitrate test, PSNT) (Magdoff et al., 1984). In the PSNT method, the N fertilizer rate is calculated based on the comparison of the soil NO_3^-–N content measured at the V_6 crop stage (0–30 cm) with regional N availability thresholds (soil N + fertilizer N) (Magdoff et al., 1984; Sainz Rozas et al., 2000). The PSNT is considered a more accurate indicator of soil N availability than PPNT as it accounts for a greater proportion of the soil N mineralized during the growing season (Magdoff et al., 1984). However, Brouder and Mengel (2003) demonstrated that PSNT underestimated corn N response in 32% of the evaluated cases ($n = 301$). Andraski and Bundy

J. Orcellet, E.E.A INTA, Ruta 34 Km 227 (2300), Rafaela, Santa Fe, Argentina; N.I. Reussi Calvo and H.R. Sainz Rozas, CONICET, Moreno 3527 (7600), Mar del Plata, Buenos Aires, Argentina; N.I. Reussi Calvo, Fertilab, Soil Testing Laboratory, Moreno 4524 (7600), Mar del Plata, Buenos Aires, Argentina; H.R. Sainz Rozas, N. Wyngaard, and H.E. Echeverría, Facultad de Ciencias Agrarias, UNMDP; Ruta 226 km 73.5, C.C. 276, (7620) Balcarce, Argentina; H.R. Sainz Rozas and H.E. Echeverría, E.E.A. INTA, Ruta 226 km 73.5, C.C. 276, (7620) Balcarce, Argentina. *Corresponding author (orcellet.juan@inta.gob.ar).

Abbreviations: JDN, Julian day number; N_{an}, ammonia released during a 7-d incubation in waterlogged conditions; N_{grain}, nitrogen concentration in grains; N_o, nitrogen mineralization potential; NP_{early}, northern Pampas (early planting date); NP_{late}, northern Pampas (late planting date); $N_{resp\%}$, corn percent yield response to nitrogen fertilization; PPNT, pre-plant nitrate nitrogen test; PSNT, pre-sidedress nitrate nitrogen test; SEP, southeastern Pampas; SOM, soil organic matter; T_m, mean temperature.

(2002) attributed this error to low N mineralization rates before soil sampling (planting to V_6 stage) caused by low temperatures.

Considering that the traditional N diagnostic methods (PPNT and PSNT) do not directly consider soil N mineralization, it is important to estimate this process to better quantity soil N availability for corn. However, the standard method to estimate N mineralization potential (N_o) (Stanford and Smith, 1972) is lengthy and laborious. Many chemical and biological laboratory indexes have been proposed to estimate N_o (Pansu and Gautheyrou, 2006; Griffin, 2008; Schomberg et al., 2009). Chemical methods, like soil extractions with NaOH (Sharifi et al., 2007), hot KCl (Gianello and Bremner, 1986), phosphate-borate (Gianello and Bremner, 1988), and the Illinois soil test analysis (Khan et al., 2001) measure the amount of specific organic N fractions that can be easily mineralized (Mulvaney et al., 2001). These methods are based on empirical approximations and their use is limited due to their complexity and/or their reduced association with the real soil N mineralization potential (Osterhaus et al., 2008; Genovese et al., 2009; Schomberg et al., 2009). Among the biological methods, the anaerobic incubation method (N_{an}) described by Keeney (1982) is simple, precise, and fast, and it is therefore suitable for soil testing labs (Soon et al., 2007). Results from N_{an} are greatly associated with N_0 (Echeverria et al., 2000; Soon et al., 2007; Schomberg et al., 2009), corn yield ($R^2 = 0.85$), and corn N uptake during the growing season ($R^2 = 0.79$) (Nyiraneza et al.. 2009). Additionally, N_{an} is sensitive to changes in soil use and management practices (Bundy and Meisinger, 1994; Soon et al., 2007; Genovese et al., 2009). Another advantage of this method as compared to other biological methods (e.g., aerobic incubations) is that due to the water saturation conditions, it is not necessary to determine the optimum water content for each soil sample. However, the mineralization rate of the labile pool quantified by N_{an} is influenced by factors such as soil texture, temperature, and water availability, which vary between areas. To overcome this inconvenience, N_{an} can be modeled together with texture and temperature to estimate the N mineralized in field conditions.

The quantification of N_{an} in soils could be a useful strategy to improve the reliability of PPNT or PSNT. For example, the use of a combined index (PPNT + N_{an}) improved the prediction of wheat (*Triticum aestivum.* L.) yield and its grain N content, as compared to the use of just PPNT (Reussi Calvo et al., 2013). For corn, PSNT critical values (value above which 94% of the maximum corn yield is achieved) were 75 and 90 kg ha^{-1} of N, for sites with high and low N_{an}, respectively (Sainz Rozas et al., 2008). However, there are currently no studies evaluating the use of a combined index (N_{an} + PPNT or PSNT) to diagnose soil N supply capacity for corn.

Considering that PPNT and PSNT only account for the initial N availability at growing stages where corn N uptake is only 10% of the total (Bender et al., 2013), N_{an} which is greatly associated with total N corn uptake (Nyiraneza et al.. 2009) would improve the performance of these traditional N diagnostic methods. The objective of our study was to evaluate if in-season N recommendations could be improved by inclusion of a N mineralization potential estimator (N_{an}) to estimate N fertilizer requirements for corn, in areas with contrasting edaphic and climatic conditions.

MATERIALS AND METHODS

Field Experiment Description

Field experiments ($n = 35$) were conducted along the Pampas region of Argentina (30°–40° S, 57°–66° W) between 2007 and 2013 (Fig. 1). The field sites were grouped into two areas with contrasting edaphic and climatic conditions: southeastern Pampas (SEP) (Typic Argiudoll; 13.5°C mean annual air temperature; 950 mm mean annual precipitation) and northern Pampas (NP) (Typic Hapludoll/Typic Argiudoll, 19.2°C mean annual air temperature; 975 mm mean annual precipitation). The soils from the SEP, where 14 experiments were conducted, are characterized by a loamy superficial texture and a high SOM concentration (50–60 g kg^{-1}). On the other hand, the soils from NP, where 20 experiments were performed, present a silty-loam texture and a lower SOM concentration (20–30 g kg^{-1}) than those in the SEP (Sainz Rozas et al., 2011). Corn was planted from Julian day number (JDN) 277–299 at SEP, while two different planting dates were evaluated at the NP area: early (NP_{early}, from JDN 271–281) and late (NP_{late}, from JDN 340–344) (13 and 5 experimental sites, respectively). The average seeding rate was 67,000; 80,000; and 73,000 plants ha^{-1} for SEP, NP_{early}, and NP_{late}, respectively. Corn crop was managed on a no-tillage system, using chemical products for weed and pest control. No irrigation was applied. Data on daily mean air temperature (T_m) and precipitation were collected from INTA (National Institute for Agricultural Technology) and SMN (National Weather Service) weather stations. The cumulative precipitation was calculated during the whole season and during the corn critical period. The average T_m was calculated from planting until the R_1 corn stage, when 75% of the total plant N is already taken by the plant (Bender et al., 2013). For the late corn planting dates, large drops in temperature during the last stage of the growing season reduce the mean temperature for the whole season. Consequently, the mean temperature does not fairly represent the temperature during the period of greater mineralization and N plant uptake.

The experimental arrangement at all sites was a randomized complete block design with three to four replications, depending on the experiment. Plot size for each treatment replication was 10 by 12 m. At each site, the effect of five N fertilizer rates was evaluated (0, 80, 120, 180, and 200 kg ha^{-1} of N). The N source was urea (46–0–0), which was broadcasted at sowing. Phosphorus (30–40 kg ha^{-1}) and S (20–25 kg ha^{-1}) fertilizers were applied to all plots to ensure a sufficient availability of these nutrients.

Soil and Plant Analysis

Soil samples were taken from each site at 0- to 20- and 0- to 60-cm depths before sowing and at a 0- to 30-cm depth at the V_6 corn stage. These samples were air-dried, ground to pass a 2-mm sieve, and all recognizable plant tissues were removed. At the surface layer (0–20 cm), particle size distribution (Bouyoucos, 1962 as modified by Gee and Bauder, 1986), SOM (Walkley and Black, 1934) and N_{an} (Bremner and Keeney, 1965) were determined. This last method consisted of incubating 10 g soil in a stoppered tube filled with water for 7 d at 40°C. After the incubation period, the resulting slurry was extracted with a 4 mol L^{-1} KCl solution and steam distillated. The ammonia in the distillate was trapped in a mixed boric acid and indicator

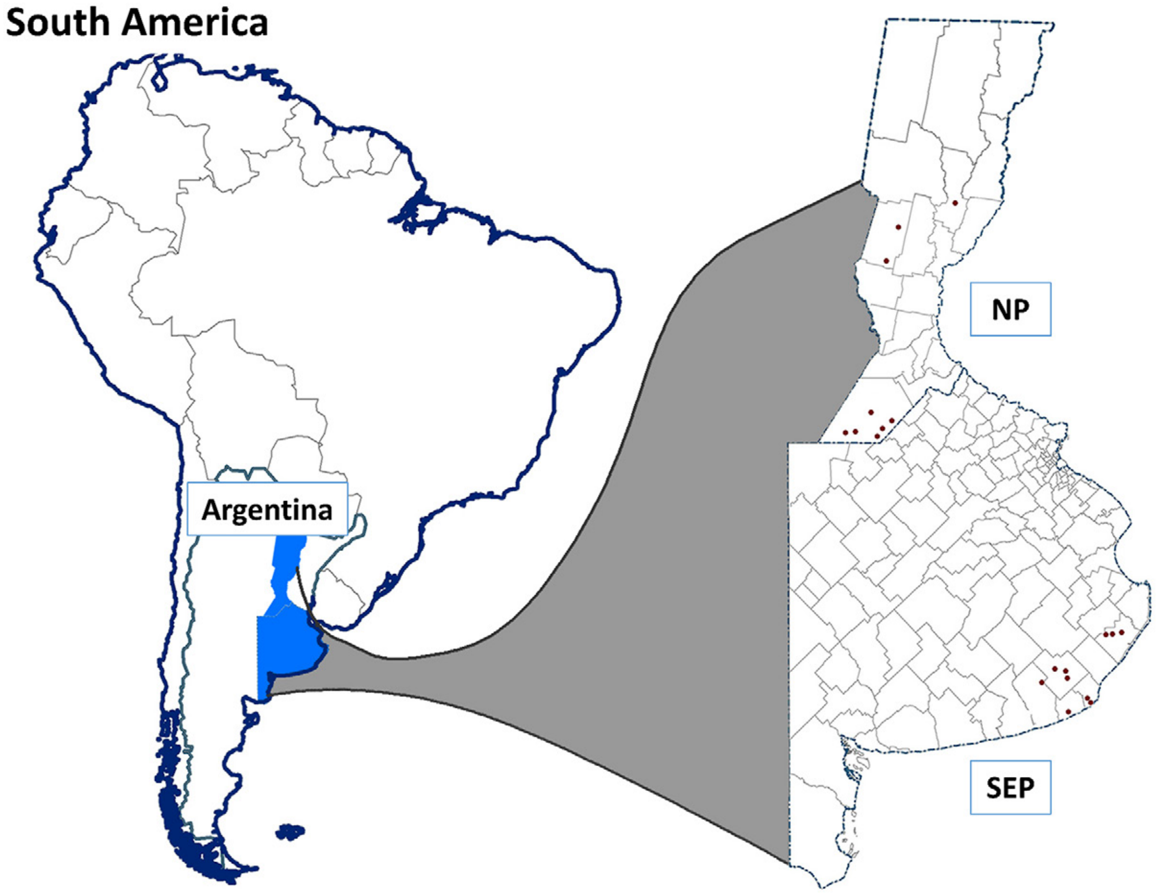

Fig. 1. Location of the experimental sites in the southeastern Pampas (SEP) and northern Pampas (NP).

solution, which was then titrated with sulfuric acid. The initial NH_4^+ content in the soil samples was subtracted from the value determined after the incubation. The NO_3–N concentration at the end of the incubation was measured by distillation after NH_4–N quantification, using Devarda alloy to reduce the NO_3–N. The quantification of NO_3–N was performed to verify the anaerobic conditions during the incubation (Canali et al., 2011). There was no significant NO_3^--N- accumulation in any of the analyzed samples. Considering that the soil particle size distribution can affect N mineralization (Six et al., 2002), we adjusted the determined N_{an} values at each region by its texture as follows: N_{an} (mg kg^{-1})/Silt + Clay (g kg^{-1}).

Soil NO_3^-–N concentration (mg kg^{-1}) was quantified by colorimetry (Keeney and Nelson, 1982) at the 0- to 60-cm samples taken at sowing (PPNT) and at the 0- to 30-cm samples taken at V_6 stage (PSNT). Soil NO_3–N content (kg ha^{-1}) was calculated assuming a 1.25 Mg m^{-3} soil bulk density (Aparicio and Costa, 2007). It must be noted that sampling depths are different for PPNT (0–60 cm), PSNT (0–30 cm) and N_{an} (0–20 cm). This is because we evaluated the N availability index at the sampling depth at which they were originally developed and calibrated (Ritchie and Hanway, 1982; Magdoff et al., 1984; Bundy and Meisinger, 1994; Sainz Rozas et al., 2008). After physiological maturity, five linear meters of three central rows were hand harvested. Threshing was performed using a stationary threshing machine, and yield was adjusted at a 140 g kg^{-1} water content. The N concentration in grains (N_{grain}) was determined by the Dumas method (Jung et al., 2003) using a TruSpec CN analyzer (LECO, 2010). Quadratic models associating N rate and yield were fit to determine the N rate at which the yield was maximum (1N rate). These models were performed for each site/year combination. The response of the crop to N fertilization ($N_{resp\%}$) was calculated as the yield difference between the control (0N) and 1N, and it was expressed as the percent increment relative to the 0N treatment.

Statistical Analysis

The normality of distribution of the data was confirmed using the Shapiro and Wilk (1965) procedure, while the homogeneity of variances was confirmed using the Levene (1960) test. Differences between edaphic variables, yield, and N_{grain} between treatments (0N and 1N) and areas/planting dates (SEP, NP_{early}, and NP_{late}) were evaluated by ANOVA using R commander routines (R Core Team, 2014). Effects were considered significant at $p < 0.05$, and the least significant difference procedure (LSD) was used to compare means if analysis of variance indicated significant effects. Linear models to predict corn grain yield at the 0N plots and $N_{resp\%}$ were fit using PPNT or PNST and N_{an} as predictors. The stepwise selection method was used to determine the best variable combination to explain yield at the 0N treatment and $N_{resp\%}$ from PPNT, PSNT, N_{an}, N_{an}/(Silt + Clay), SOM, texture, precipitations, and temperature. This selection process was used to determine the best predictive model at each individual region and also considering all regions jointly. For all statistical analysis, each site and year combination was analyzed separately (site results were not averaged between years).

Table 1. Climatic and edaphic variables at the southeastern Pampas (SEP), and at the northern Pampas at two different planting dates: early and late (NP_{early} and NP_{late}, respectively) in different corn field studies carried out from 2007 to 2013. Mean values ± standard deviation.†

Region	Pp	Pp at Cp	T_m R_1	Clay	Silt	SOM	N_{an}	PPNT	PSNT
	mm		°C	g kg^{-1}			mg kg^{-1}	kg ha^{-1}	mg kg^{-1}
SEP	571 ± 82c	165 ± 41b	19 ± 1c	220 ± 30a	310 ± 70a	60 ± 5a	70 ± 12a	79 ± 28a	12 ± 4a
NP_{early}	636 ± 34b	149 ± 7b	22 ± 1b	80 ± 30b	180 ± 30b	25 ± 6b	30 ± 8b	69 ± 12b	6 ± 2b
NP_{late}	723 ± 0a	228 ± 0a	26 ± 0a	120 ± 30c	210 ± 70b	29 ± 5b	29 ± 7b	66 ± 5b	12 ± 4a

† N_{an}, anaerobically incubated N; Pp, precipitations during the season; Pp at Cp, precipitations during corn critical period; PPNT, NO_3^-–N determined at planting at a 0- to 60-cm depth; PSNT, NO_3^-–N determined at corn V_6 stage at a 0- to 30-cm depth; SOM, soil organic matter; T_m R_1, mean temperature from planting to corn R_1 stage.

RESULTS AND DISCUSION

Precipitations during the 2007 to 2013 growing season were, in general, sufficient to satisfy the water demand of the crop (between 530 and 575 mm) (Andrade and Gardiol, 1995; Castellarín et al., 2010) (Table 1). The T_m at the R_1 stage (Ritchie and Hanway, 1982) differed between sites and planting dates, and its order of magnitude was $NP_{late} > NP_{early} >$ SEP (Table 1).

The SOM content was greater at SEP as compared to NP (60 and 27 g kg^{-1}, respectively). We observed a strong correlation between SOM and soil clay content (SOM (g kg^{-1}) = 7.4 + 2.3 × clay content (%), $R^2 = 0.79$, $p < 0.0001$), which is likely explained by the greater capacity of soil fines to physically protect SOM from decomposition (Six et al., 2002). However, the lower SOM content at NP relative to SEP can be also a consequence of the SOM depletion caused by the long farming history and the use of aggressive tillage systems in the NP (Sainz Rozas et al., 2011). This difference in SOM between areas resulted in contrasting N_{an} values (70 mg kg^{-1} for SEP and 30 mg kg^{-1} for NP_{early} and NP_{late}), as both variables were strongly correlated [N_{an} (mg kg^{-1}) = −0.81 + 1.2 × SOM (g kg^{-1}), $R^2 = 0.85$, $p < 0.0001$].

The mean NO_3^-–N at pre-planting (PPNT) was 79 kg ha^{-1} at SEP, 69 kg ha^{-1} at NP_{early} and 66 kg ha^{-1} at NP_{late} (Table 1). As described by Reussi Calvo et al. (2013) for wheat (*Triticum aestivum* L.), there was no association between PPNT and N_{an}. This is because the soil inorganic N content does not only depend on the size of the mineralizable N pool, but on the balance between N inputs and losses (Genovese et al., 2009; Divito et al., 2011). This lack of correlation between N_{an} and PPNT reinforces the validity of developing a combined index using these two variables (PPNT + N_{an}). The PSNT value was similar for SEP and NP_{late}, but lower for NP_{early} (Table 1). Even though PSNT is meant to account for part of the N mineralized during the crop growing season (Meisinger et al., 1992), there was no association between N_{an} and PSNT.

Corn yield in the control plots (0N) varied between areas and planting dates in the order $NP_{late} > NP_{early} =$ SEP (Fig. 2). The differences in rainfall and N mineralization can explain these differences in yield at the 0N plots. Corn yield response to N fertilization was 2076 ± 672 kg ha^{-1} for NP_{early} ≥ 1677 ± 625 kg ha^{-1} for SEP ≥ and 999 ± 670 kg ha^{-1} for NP_{late}. The N_{grain} content at 0N and 1N was greater at the SEP region than in the other regions, coinciding with the lower corn grain yield we observed (Fig. 2).

We determined a significant but weak relationship between PPNT and corn yield (R^2 ranging from 0.20–0.36) when analyzing each region/sowing date separately (Table 2). This low association coincides with that described by other authors (Magdoff et al., 1984; Alvarez et al., 2001). However, when integrating all regions/planting dates in a single model, PPNT did not significantly predict corn yield, probably due to the

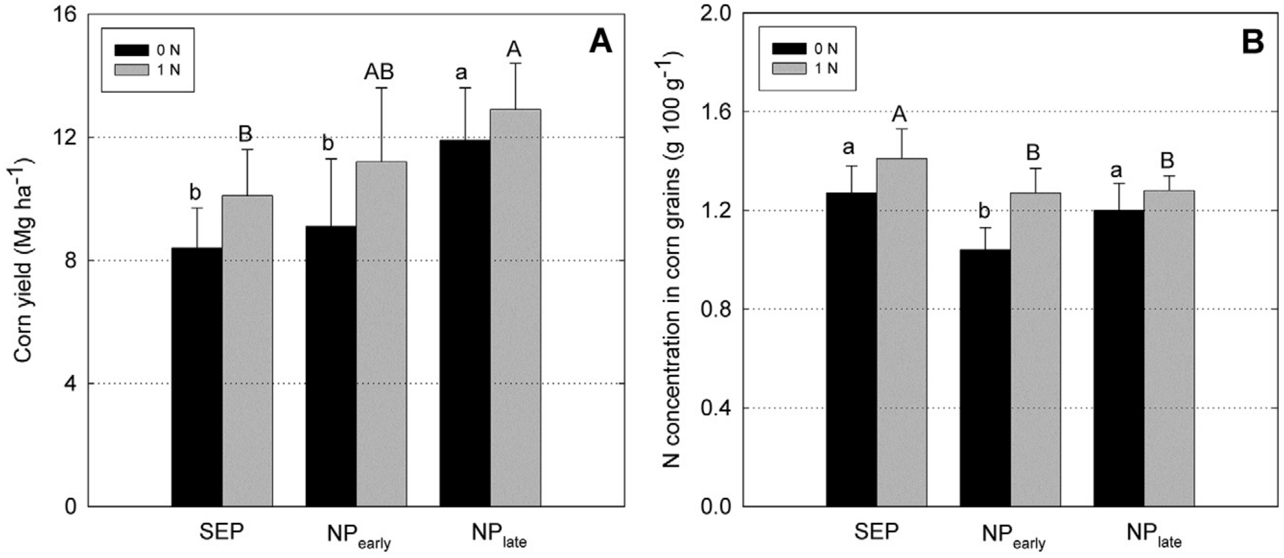

Fig. 2. (A) Corn grain yield and (B) N concentration in un-fertilized plots (0N) or plots fertilized with the optimum N rate (1N) at the southeastern Pampas (SEP), and at the northern Pampas at two different planting dates: early and late (NP_{early} and NP_{late}, respectively) in different corn field studies performed from 2007 to 2013. Means ± standard deviation. Different lowercase letters indicate significant differences ($p < 0.05$) between regions for the 0N treatment, as stablished by LSD test, while capital letters indicate differences for the 1N treatment.

different amount of mineralized N between locations (Table 2). These results indicate that, as stated by Sainz Rozas et al. (2008), PPNT is not a reliable index to predict corn yield.

The incorporation of N_{an} to the PPNT model to predict yield at the 0N treatment increased its determination coefficient values to 0.49, 0.69, and 0.72, with a partial contribution from N_{an} to the model of 29, 46, and 36% at SEP, NP_{early}, and NP_{late}, respectively (Table 2). The contribution of all other evaluated variables [Nan/(Silt + Clay), SOM, texture, precipitations, and temperature] to the model was not significant. The lesser contribution of N_{an} to the PPNT model at the SEP was probably a consequence of the lower temperature and precipitation in this area, which reduce the N mineralization rate and its relative contribution to corn N nutrition (Echeverría et al., 2014, Reussi Calvo et al., 2014).

The relationship between PSNT and yield at the 0N treatment was significant for the SEP and NP_{early} regions (Table 2), where PSNT explained 32 and 72% of the yield variability, respectively. A similar association between PSNT and yield has been described by other authors (Salvagiotti et al., 2001; Andraski and Bundy, 2002; Ma and Wu, 2008; Sainz Rozas et al., 2008). The different predictive capacity of PSNT between regions is a consequence of the different T_m between planting and V_6 stages (18°C at SEP and 21°C at NP_{early}). The greater the temperature during that period, the greater is the amount of mineralizable N accounted for by the PSNT method (Andraski and Bundy, 2002), reducing the necessity of an additional method to estimate N mineralization. The lack of association between PSNT and yield at NP_{late} can be a consequence of the heavy rainfall before soil sampling observed in this region (70–80 mm, data not shown), that may have leached the NO_3^- below the 30-cm sampling depth (Magdoff, 1991). As for PPNT, there was no relationship between PSNT and corn yield when integrating all regions/planting dates (Table 2). In summary, we observed a greater yield predictive capacity when using PSNT, in comparison to PPNT. This is because the former takes into account part of the N mineralized during the growing season, and it quantifies the N availability during the stage of maximum N plant uptake (Uhart and Andrade, 1995; Sainz Rozas et al., 2004).

The incorporation of N_{an} to the PSNT model explained 69% of the yield variation at the 0N treatment at SEP and 77% at NP_{early} (the model was not significant at NP_{late}), with a partial contribution from N_{an} of 37 and 5%, respectively (Table 2). The fact that N_{an} contributed significantly to the PSNT model suggests that the N mineralized from planting to V_6 is not sufficient to predict the total N mineralized throughout the whole corn growing season. This can be a consequence of the limited N mineralization rate during the planting to V_6 stage caused by the relatively low T_m (Magdoff, 1991; Andraski and Bundy, 2002), which underestimates the real mineralization occurring during the whole growing season. The contribution of N_{an} to the PSNT model was greater at the SEP as compared to NP_{early} due to the lower T_m during the planting to V_6 stage in this area. Consequently, even though the contribution of N_{an} to the PSNT was significant for both SEP and NP_{early}, our results

Table 2. Parameters from models to predict corn grain yield in un-fertilized plots (0N) (kg ha^{-1}) as a function of soil NO_3^-–N content before planting (PPNT) (kg ha^{-1}) (0–60 cm) or at corn V_6 stage (PSNT) (mg kg^{-1}) (0–30 cm) and incubated N in anaerobic conditions (N_{an}) (mg kg^{-1}). Individual models were performed for different areas: southeastern Pampas (SEP) and northern Pampas at two different planting dates: early and late (NP_{early} and NP_{late}, respectively) in different corn field studies carried out from 2007 to 2013. The global model describes all the areas/sowing dates jointly.

		Corn yield prediction models							
		PPNT				PSNT			
Region	Variable	PV†	*p* value	Partial R^2	R^2	PV	*p* value	Partial R^2	R^2
SEP	Intercept	6889.0	–	–	0.20	6875.4	–	–	0.32
	NO_3^-–N	19.5	0.002	–		139.6	0.015	–	
	Intercept	3471			0.49	3024	–	–	0.69
	NO_3^-–N	13.0	0.002	0.20		98	0.004	0.32	
	N_{an}	56.0	0.001	0.29		59	0.001	0.37	
NP_{early}	Intercept	3455.0	–	–	0.23	3425	–	–	0.72
	NO_3^-–N	86.9	0.01	–		832	0.001	–	
	Intercept	–75.3	–	–	0.69	2416.2	–	–	0.77
	NO_3^-–N	51.7	0.020	0.23		528.8	0.022	0.72	
	N_{an}	192.7	0.001	0.46		99.8	0.099	0.05	
NP_{late}	Intercept	220.4	–	–	0.36	–	ns‡	–	–
	NO_3^-–N	176.9	0.01	–					
	Intercept	3786.7	–	–	0.72	–	ns	–	–
	NO_3^-–N	45.6	0.001	0.36					
	N_{an}	172.9	0.001	0.36					
Global	Intercept	8284	–	–	0.02	7837	–	–	0.09
	NO_3^-–N	13.6	0.192	–		160	0.016	–	
	Intercept	8768.4	–	–	0.07	8695.85	–	–	0.14
	NO_3^-–N	22.9	0.182	0.02		212.98	0.002	0.09	
	N_{an}	–22.7	0.174	0.05		–28.95	0.015	0.05	

† PV, parameter value.

‡ ns, the model was not significant at a 0.05 significance level.

suggest that the benefit of incorporating N_{an} to the PSNT model is lower in areas with high temperatures before soil sampling. The incorporation of other variables to the predictive model was not significant according to the Stepwise selection process.

The PPNT index explained 14, 14, and 22% of the $N_{resp\%}$ variation at the SEP, NP_{early}, and NP_{late}, respectively (Table 3). These low coefficients of determination are similar to those we observed for yield at the 0N plots, and they coincide with those described by other authors (Calviño and Echeverría, 2003; Ma and Wu, 2008; Sainz Rozas et al., 2008). The relationship between PPNT and $N_{resp\%}$ was not significant when jointly analyzing all regions/sampling dates. On the other hand, PSNT explained 23 and 70% of the $N_{resp\%}$ at the SEP and NP_{early} (Table 3). As for corn grain yield at the N0 treatment, we observed that the capacity of PSNT to predict $N_{resp\%}$ was greater than that of PPNT.

The PPNT + N_{an} model had a greater capacity to estimate $N_{resp\%}$ than PPNT (Table 3). The contribution of N_{an} to the model was 12, 35, and 41% for SEP, NP_{early}, and NP_{late}, respectively.

The N_{an} contribution at the SEP region was similar to that previously described in the same area for wheat (11%) (Reussi Calvo et al., 2013). Again, the effect of T_m over N mineralization rates can explain the different contribution of N_{an} to the PPNT model. Greater T_m values caused a greater N mineralization (Orcellet, 2015), resulting in a lower predictive

Table 3. Parameters from models to predict corn response to N fertilization (%) ($N_{resp\%}$) as a function of soil NO_3^-–N content before planting (PPNT) (kg ha^{-1}) (0–60 cm) or at corn V_6 stage (PSNT) (mg kg^{-1}) (0–30 cm) and incubated N in anaerobic conditions (N_{an}) (mg kg^{-1}). Individual models were performed for different areas: southeastern Pampas (SEP) and northern Pampas at two different planting dates: early and late (NP_{early} and NP_{late}, respectively) in different corn field studies carried out from 2007 to 2013. The global model describes all the areas/sowing dates jointly.

		Corn N response prediction models							
		PPNT				PSNT			
Region	Variable	PV†	*p* value	Partial *R*^{2}	*R*^{2}	PV	*p* value	Partial *R*^{2}	*R*^{2}
SEP	Intercept	22.22	–	–	0.14	21.6	–	–	0.23
	NO_3^-–N	–0.05	0.031	–		–0.24	0.014	–	
	Intercept	28.5	–	–	0.26	28.6	–	–	0.39
	NO_3^-–N	–0.03	0.023	0.14		–0.21	0.015	0.23	
	N_{an}	–0.12	0.041	0.12		–0.1	0.021	0.16	
NP_{early}	Intercept	38.8	–	–	0.14	43.5	–	–	0.70
	NO_3^-–N	–0.23	0.076	–		–3.03	0.001	–	
	Intercept	47.5	–	–	0.49	46.40	–	–	0.70
	NO_3^-–N	–0.12	0.029	0.14		–2.16	0.022	–	
	N_{an}	–0.54	0.001	0.35		–0.30	0.218	–	
NP_{late}	Intercept	45.8	–	–	0.22	–	ns‡	–	–
	NO_3^-–N	–0.53	0.088	–					
	Intercept	31.1	–	–	0.63	–	ns	–	–
	NO_3^-–N	–0.001	0.025	0.22					
	N_{an}	–0.69	0.005	0.41					
Global	Intercept	25.00491	–	–	0.06	25.85	–	–	0.19
	NO_3^-–N	–0.08755	0.05	–		–0.73	0.001	–	
	Intercept	25.74178	–	–	0.08	25.71	–	–	0.19
	NO_3^-–N	–0.06923	0.109	0.06		–0.74	0.001	0.19	
	N_{an}	–0.04118	0.260	0.02		0.005	0.904	–	

† PV, parameter value.

‡ ns, the model was not significant at a 0.05 significance level.

Table 4. Parameters from models to predict corn grain yield in un-fertilized plots (0N) (kg ha^{-1}) and response to N fertilization ($N_{resp\%}$) (%) from the soil nitrate nitrogen (NO_3^-–N) content before planting (PPNT) (kg ha^{-1}) (0–60 cm) or at corn V_6 stage (PSNT) (mg kg^{-1}) (0–30 cm), the mean air temperature at the R_1 corn stage (T_m at R_1) (°C), and the ratio between the incubated N in anaerobic conditions and the silt + clay content (N_{an}/Silt + Clay). The data from three different Pampas regions/planting dates were analyzed jointly.

	Yield at 0N				$N_{resp\%}$			
Variable	PV†	*p* value	Partial *R*^{2}	*R*^{2}	PV	*p* value	Partial *R*^{2}	*R*^{2}
Intercept	–6,615	–	–	0.47	56.33	–	–	0.23
PPNT	17	0.056	0.02		–0.08	0.077	0.04	
T_m at R_1	569	0.001	0.32		–1.16	0.010	0.08	
N_{an}/(Silt + Clay)	20,414	0.001	0.13		–5.83	0.002	0.12	
Intercept	–6,207	–	–	0.53	63.88	–	–	0.42
PSNT	163	0.001	0.10		–0.69	0.001	0.17	
T_m at R_1	540	0.001	0.36		–1.33	0.001	0.13	
N_{an}/(Silt + Clay)	18,070	0.005	0.07		–7.98	0.002	0.12	

† PV, parameter value.

capacity of PPNT and a greater contribution of N_{an} to the model. On the other hand, the incorporation of N_{an} to the PSNT model improved the estimation of $N_{resp\%}$ only at SEP. At NP_{early} and NP_{late}, where PSNT was sufficient to predict $N_{resp\%}$ (Table 3), the N_{an} term of the model was not significant.

Finally, we evaluated incorporating edaphic and climatic variables to the model to estimate yield at N0 and $N_{resp\%}$. Incorporation of these variables was to account for the differences among regions not only in their mineralization potential but in their mineralization rate. From the results of the stepwise analysis, the only edaphic and climatic variables that significantly affected the model were the N_{an}/(Silt + Clay) ratio and T_m at the corn R_1 stage (Table 4). The addition of these variables to the PPNT models to estimate yield at the N0 treatment and $N_{resp\%}$ increased their determination coefficient from 0.07 to 0.47 and from 0.08 to 0.23, respectively (Tables 2, 3, and 4). For PSNT, the determination coefficient was increased from 0.14 to 0.53 and from 0.19 to 0.42 when estimating N0 and $N_{resp\%}$, respectively (Tables 2, 3, and 4).

The validity of the combined models presented in Table 4 is limited due to the reduced number of sites-years analyzed in our study, and it would be necessary to validate them with data from other studies. However, our results demonstrate that it is possible to improve the predictive capacity of the traditional methods (PPNT and PSNT) by accounting for N mineralization (estimated by Nan and edaphic-climatic variables). It would be interesting to evaluate N_{an} not only in soil-based analysis, but in newly developed N management tools as computer-based simulation models and sensor-based N fertilizer recommendations (Thompson et al., 20115; Sela et al., 2016).

CONCLUSION

Adding the N_{an} indicator to the traditional methods of diagnosing fertilizer N requirements (e.g., PPNT and PSNT) improved the predictions of corn response to N fertilization in areas with similar edaphic-climatic characteristics. Moreover, corn yield predictions were stronger when the model combined PPNT or PSNT scores with a measure of N mineralization (estimated from N_{an}, texture, and temperature), independent of the geographical region. Our method can provide better estimates of soil N availability for corn in humid temperate agroecosystems, although it remains to be validated for a wider range of edaphic-climatic conditions. An updated model of soil N supply based on our approach is suitable for determining the optimal N fertilizer rate for corn that will minimize the economic cost and environmental impact of N fertilization.

ACKNOWLEDGMENTS

This work was funded by the research projects UNMdP 447/14 and INTA PNSUELO-1134024, and it was supported by Fertilab (Plant and Soil testing Lab) and INTA Balcarce experimental station. We would like to thank Dr. Kate Cassity-Duffey from the University of Georgia for reviewing the use of English in our manuscript.

REFERENCES

Alvarez, C.R., R. Alvarez, and H.S. Steinbach. 2001. Predictions of available nitrogen content in soil profile depth using available nitrogen concentration in surface layer. Commun. Soil Sci. Plant Anal. 32:759–769. doi:10.1081/CSS-100103907

Andrade, F.H., and J. Gardiol. 1995. Drought and crop production of corn and soybeans sunflower. Boletín técnico 132. (In Spanish.) EEA INTA, Balcarce, Argentina.

Andraski, T.W., and L.G. Bundy. 2002. Using the presidedress soil nitrate test and organic nitrogen crediting to improve corn nitrogen recommendations. Agron. J. 94:1411–1418. doi:10.2134/agronj2002.1411

Aparicio, V.C., and J.L. Costa. 2007. Soil quality indicators under continuous cropping systems in the Argentinean Pampas. Soil Tillage Res. 96:155–165. doi:10.1016/j.still.2007.05.006

Bender, R.R., J.W. Haegele, M.L. Ruffo, and F.E. Below. 2013. Modern corn hybrids' nutrient uptake patterns. Better Crops Plant Food 97:7–10.

Bouyoucos, G.J. 1962. Hydrometer method for making particle size analysis in soils. Agron. J. 54:464–465. doi:10.2134/agronj1962.00021962005400050028x

Bremner, J., and D. Keeney. 1965. Steam distillation methods for determination of ammonium, nitrate and nitrite. Analytica Chim. Acta 32:485–495. doi:10.1016/S0003-2670(00)88973-4

Brouder, S.M., and D.B. Mengel. 2003 The presidedress soil nitrate test for improving N management in corn. AY-314-W. Purdue Univ. Coop. Ext. Serv., Purdue, IN.

Bundy, L.G., and J.J. Meisinger. 1994. Nitrogen availability indices. In: R.W. Weaver et al., editors, Methods of soil analysis. Part 2. SSSA Book Ser. 5. SSSA, Madison, WI. p. 951–984.

Calviño, P.A., and H.E. Echeverría. 2003. Incubación anaeróbica de suelo como ayuda al diagnóstico de la respuesta a nitrógeno en maíz bajo siembra directa. (In Spanish, with English abstract.) Cienc. Suelo 21:24–29.

Canali, S., E. Di Bartolomeo, F. Tittarelli, F. Montemurro, V. Verrastro, and D. Ferri. 2011. Comparison of different laboratory incubation procedures to evaluate nitrogen mineralization in soils amended with aerobic and anaerobic stabilized organic materials. J. Food Agric. Environ. 9:540–546.

Castellarín, J., F. Ferraguti, and J. Andriani. 2010. Environmental characterization and determination of the yield of maize during the 2009/10 campaign in Oliveros (Santa Fe). (In Spanish.) To improve production 44. INTA, Oliveros, Argentina.

Divito, G.A., H.R. Sainz Rozas, H.E. Echeverría, G.A. Studdert, and N. Wyngaard. 2011. Long term nitrogen fertilization: Soil property changes in an Argentinean Pampas soil under no tillage. Soil Tillage Res. 114:117–126. doi:10.1016/j.still.2011.04.005

Echeverría, H.E., H.R. Sainz Rozas, and P.A. Barbieri. 2014. Corn and sorghum. In: H.E. Echeverría and F.O. García, editors, Soil fertility and fertilization of crops. (In Spanish.) Ediciones INTA, Buenos Aires, Argentina. p. 435–478.

Echeverria, H.E., N. San Martin, and R. Bergonzi. 2000. Rapid methods for assessing potentially mineralizable soil nitrogen. (In Spanish, with English abstract.) Cienc. Suelo 18:9–16.

Gee, G.W., and J.W. Bauder. 1986. Particle size analysis. In: G.R. Blake and K.H. Hartge, editors, Methods of aoil analysis. Part 1. Physical and mineralogical methods. 2nd ed. Agronomy Monogr. 9. ASA and SSSA, Madison, WI. p. 383–411.

Genovese, M. F., H.E. Echeverría, G.A. Studdert, and H. R. Sainz Rozas. 2009. Nitrógeno de amino-azúcares en suelo: Calibración y relación con el nitrógeno incubado anaeróbico. (In Spanish, with English abstract.) Cienc. Suelo 27:225–236.

Gianello, C., and J.M. Bremner. 1986. Comparison of chemical methods of assessing potentially available organic nitrogen in soil. Commun. Soil Sci. Plant Anal. 17:215–236. doi:10.1080/00103628609367709

Gianello, C., and J.M. Bremner. 1988. A rapid steam distillation method of assessing potentially available organic nitrogen in soil. Commun. Soil Sci. Plant Anal. 19:1551–1568. doi:10.1080/00103628809368034

Griffin, T.S. 2008. Nitrogen availability. In: J.S. Schepers and W.R. Raun, editors, Nitrogen in agricultural systems. Agron. Monogr. 49. ASA, CSSA, and SSSA, Madison, WI. p. 613–646. doi:10.2134/agronmonogr49.c15

Jung, S., D.A. Rickert, N.A. Deak, E.D. Aldin, J. Recknor, L.A. Johnson, and P.A. Murphy. 2003. Comparison of Kjeldahl and Dumas methods for determining protein contents of soybean products. J. Am. Oil Chem. Soc. 80:1169–1173. doi:10.1007/s11746-003-0837-3

Keeney, D.R. 1982. Nitrogen-availability indices. In: A.L. Page et al., editors, Methods of soil analysis. Part 2. Chemical and microbiological properties. Agron. Monogr. 9. ASA and SSSA, Madison, WI. p. 711–733.

Keeney, D.R., and D.W. Nelson. 1982. Nitrogen-inorganic forms. In: A.L. Page et al., editors, Methods of soil analysis. Part 2. Chemical and microbiological properties. Agron. Monogr. 9. ASA and SSSA, Madison, WI. p. 643–698.

Khan, S.A., R.L. Mulvaney, and R.G. Hoeft. 2001. A simple soil test for detecting sites that are nonresponsive to nitrogen fertilization. Soil Sci. Soc. Am. J. 65:1751–1760. doi:10.2136/sssaj2001.1751

LECO. 2010. Organic application notes. LECO, St. Joseph, MI. http://www.leco.com/resources/application_note_subs/organic_application_notes.htm (accessed 30 Nov. 2015).

Levene, H. 1960. Robust tests for equality of variances. In: I. Olin, editor, Contributions to probability and statistics: Essays in honour of Harold Hotelling. Stanford Univ. Press, Standford, CA. p. 278–292.

Ma, B.L., and T.Y. Wu. 2008. Plant-available nitrogen in the soil: Relationships between pre-plant and pre-sidedress nitrate tests for corn production. J. Plant Nutr. Soil Sci. 171:458–465. doi:10.1002/jpln.200700091

Magdoff, F.R. 1991. Understanding the Magdoff pre-sidedress nitrate test for corn. J. Prod. Agric. 4:297–305. doi:10.2134/jpa1991.0297

Magdoff, F.R., D. Ross, and J. Amadon. 1984. A soil test for nitrogen availability to maize. Soil Sci. Soc. Am. J. 48:1301–1304. doi:10.2136/sssaj1984.03615995004800060020x

Meisinger, J.J., F.R. Magdoff, J.S. Schepers, K.L. Wells, and M.J. Bitzer. 1992. Nitrogen management in the no fertilizer needs for maize in humid regions: Underlying principles. In: B.R. Bock and K.R. Kelley, editors, Predicting N fertilizer needs for maize in humid regions. Bull. Y-226. Natl. Fertilizer Development Ctr. TVA, Muscle Shoals, AL. p. 8–26.

Mulvaney, R.L., S.A. Khan, R.G. Hoeft, and H.M. Brown. 2001. A soil organic nitrogen fraction that reduces the need for nitrogen fertilization. Soil Sci. Soc. Am. J. 65:1164–1172. doi:10.2136/sssaj2001.6541164x

Nyiraneza, J., A. N'Dayegamiye, M.H. Chantigny, and M.R. Laverdière. 2009. Variations in corn yield and nitrogen uptake in relation to soil attributes and nitrogen availability indices. Soil Sci. Soc. Am. J. 73:317–327. doi:10.2136/sssaj2007.0374

Orcellet, J.M. 2015. Contribution of nitrogen incubated anaerobically the diagnosis of nitrogen in corn. Magister thesis. Univ. Nacional de Mar del Plata, Balcarce, Argentina.

Osterhaus, J.T., L.G. Bundy, and T.W. Andraski. 2008. Evaluation of the Illinois soil nitrogen test for predicting corn nitrogen needs. Soil Sci. Soc. Am. J. 72:143–150. doi:10.2136/sssaj2006.0208

Pansu, M., and J. Gautheyrou, editors. 2006. Organic forms of nitrogen, mineralizable nitrogen (and carbon). In: M. Pansu and J. Gautheyrou, editors, Handbook of soil analysis-Mineralogical, organic and inorganic methods. Springer, Berlin. p. 497–547. doi:10.1007/978-3-540-31211-6_14

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org (accessed 30 Nov. 2015).

Reussi Calvo, N.I., H.E. Echeverría, H.R. Sainz Rozas, A. Berardo, and N. Diovisalvi. 2014. Can a soil mineralization test improve wheat and corn nitrogen diagnosis? Better Crops Plant Food 98:12–14.

Reussi Calvo, N.I., H.R. Sainz Rozas, H.E. Echeverría, and A. Berardo. 2013. Contribution of anaerobically incubated nitrogen to the diagnosis of nitrogen status in spring wheat. Agron. J. 105:321–328. doi:10.2134/agronj2012.0287

Ritchie, S.W., and J.J. Hanway. 1982. How a corn plant develops. Spec. Rep. no. 48. Iowa State Univ. of Sci. and Technol. Coop. Ext. Serv., Ames.

Sainz Rozas, H.R., P.A. Calviño, H.E. Echeverría, P.A. Barbieri, and M. Redolati. 2008. Contribution of anaerobically mineralized nitrogen to the reliability of planning or presidedress soil nitrogen test in maize. Agron. J. 100:1020–1025. doi:10.2134/agronj2007.0077

Sainz Rozas, H., H. Echeverría, and H.P. Angelini. 2011. Organic carbon and ph levels in agricultural soils of the pampa and extra-pampean regions of Argentina. (In Spanish, with English abstract.) Cienc. Suelo 29:29–37.

Sainz Rozas, H.R., H.E. Echeverría, and P.A. Barbieri. 2004. Nitrogen balance as affected by application time and nitrogen fertilizer rate in irrigated no-tillage maize. Agron. J. 96:1622–1631. doi:10.2134/agronj2004.1622

Sainz Rozas, H. , H. E. Echeverría, G.A. Studdert, and G. Dominguez. 2000. Evaluation of the presidedress soil nitrogen test for no-tillage maize fertilized at planting. Agron. J. 92:1176–1183. doi:10.2134/agronj2000.9261176x

Salvagiotti, F., H. Pedrol, and J. Castellarín. 2001. Diagnóstico de la fertilización nitrogenada en maíz. II. Relación entre la respuesta en rendimiento y la concentración de N-NO$_3$ en el suelo en el estadio V4-V6. (In Spanish.) To improve production 20. INTA, Oliveros, Argentina.

Schomberg, H.H., S. Wietholter, T.S. Griffin, D.W. Reeves, M.L. Cabrera, D.M. Endale et al. 2009. Assessing indices for predicting potential nitrogen mineralization in soils under different management systems. Soil Sci. Soc. Am. J. 73:1575–1586. doi:10.2136/sssaj2008.0303

Sela, S., H.M. van Es, B.N. Moebius-Clune, R. Marjerison, J. Melkonian, D. Moebius-Clune et al. 2016. Adapt-N outperforms grower-selected nitrogen rates in Northeast and Midwestern United States strip trials. Agron. J. 108:1726–1734. doi:10.2134/agronj2015.0606

Shapiro, S.S., and M.B. Wilk. 1965. An analysis of variance test for normality (complete samples). Biometrika 52:591–611. doi:10.1093/biomet/52.3-4.591

Sharifi, M., B.J. Zebarth, D.L. Burton, C.A. Grant, and J.M. Cooper. 2007. Evaluation of some indices of potentially mineralizable nitrogen in soil. Soil Sci. Soc. Am. J. 71:1233–1239. doi:10.2136/sssaj2006.0265

Six, J., R. Conant, E. Paul, and K. Paustian. 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant Soil 241:155–176. doi:10.1023/A:1016125726789

Soon, Y.K., A. Haq, and M.A. Arshad. 2007. Sensitivity of nitrogen mineralization indicators to crop and soil management. Commun. Soil Sci. Plant Anal. 38:2029–2043. doi:10.1080/00103620701548688

Stanford, G., and S. Smith. 1972. Nitrogen mineralization potentials of soils. Soil Sci. Soc. Am. J. 36:465–472. doi:10.2136/sssaj1972.03615995003600030029x

Uhart, S.A., and F.H. Andrade. 1995. Nitrogen deficiency in maize: I, Effects on crop growth, development, dry matter, partitioning, and kernel set. Crop Sci. 35:1376–1383. doi:10.2135/cropsci1995.0011183X003500050020x

Walkley, A., and Y. Black. 1934. An examination of the Degtjareff method for determining soil organic matter and proposed codification of the chromic acid titration method. Soil Sci. 37:29–38. doi:10.1097/00010694-193401000-00003