Contribution of Anaerobically Incubated Nitrogen to the Diagnosis of Nitrogen Status in Spring Wheat

Nahuel I. Reussi Calvo,* H. Sainz Rozas, H. Echeverría, and A. Berardo

ABSTRACT

Current wheat (*Triticum aestivum* L.) N fertility diagnosis models do not take into account organic N provided by mineralization. Anaerobically incubated N (Nan) could contribute to assess this N pool for crops. The aim of this research was to assess the Nan contribution to spring wheat yield without added N (GY_{0N}), to grain N export (GNE), and to N fertilization response. A total of 28 N fertilization experiments were conducted in 2006, 2008, 2009, 2010, and 2011 in southeastern Buenos Aires Province (Argentina). At sowing, soil organic matter (SOM) content, Nan, and NO_3^-–N availability varied between 44 and 68 g SOM kg^{-1}, 34 and 94 mg NH_4^+–N kg^{-1}, and 39 and 130 kg NO_3^-–N ha^{-1}, respectively. Grain yields and average protein contents without N added were 3450, 4330, 5020, 5288 and 6262 kg ha^{-1}, and 116, 97, 95, 91, and 90 g kg^{-1}, for 2006, 2008, 2009, 2010, and 2011, respectively. Initial NO_3^-–N availability explained only 24% of GY_{0N} variation, but R^2 increased to 66% when Nan was integrated into the model. Soil NO_3^-–N content and Nan explained 58% of GNE variation, with a higher partial contribution of Nan to GNE than to GY_{0N} (51 and 41%, respectively). A model was developed to predict the response to N ($RN = -625.7 + 7.2Pp - 31.6Nan + 0.28GY_{0N}Pp$, where Pp is total precipitation from July to December; $R^2 = 0.58$). Soil Nan determination and initial NO_3^-–N content should be taken into account together when assessing spring wheat N needs.

Current N fertilizer recommendations for spring wheat are mainly based on the determination of soil NO_3^-–N content (0–60 cm deep) at sowing (Calviño et al., 2002; Barbieri et al., 2009). To use it, different N availability thresholds (soil + fertilizer) have been suggested, which vary according to the area, farming systems, and crop yield objective (Barbieri et al., 2009; García et al., 2010). A recent study conducted with different wheat genotypes has shown that critical levels of N availability for applications at sowing and at tillering were 174 and 152 kg ha^{-1}, respectively (Barbieri et al., 2012). These kinds of simplified models do not explicitly take into account N supply through mineralization, however, which represents one of the main N sources for crops (Rice and Havlin, 1994; Campbell et al., 2008), especially in soils with high SOM content (Echeverría and Ferrari, 1993). Under medium- to high-yielding conditions, mineralization of N from soil organic pools during the growing season meets 30% of wheat (Gonzalez Montaner et al., 1997) and 60% of corn (*Zea mays* L.) (Steinbach et al., 2004) demands for N. It is worth mentioning that soil potential N mineralization is affected by cropping history (years from the last pasture), soil management practices, and weather conditions (Genovese et al., 2009; Studdert and Echeverría, 2006; Fabrizzi et al., 2003; Cozzoli et al., 2010; Alvarez and Steinbach, 2011).

The Pampa region in Argentina (30–40°S and 57–66°W) is known as one of the most important world grain-producing areas (Satorre and Slafer, 1999), with wheat, corn, and soybean [*Glycine max* (L.) Merr.] as its main crops. Southeastern Buenos Aires Province is one of the major wheat production subregions, with a cropped area of 0.7 million ha and a production of 2.5 million Mg (Fig. 1). Recently, farming activity has intensified in all the Pampa region and particularly in southeastern Buenos Aires Province, thus reducing the SOM content (Sainz Rozas et al., 2011). At the same time, the adoption of conservation tillage systems (mainly no-till [NT]) and the shorter fallow periods associated with a higher frequency of soybean as the preceding crop has affected the soil N supply by mineralization to spring wheat (Studdert and Echeverría, 2000; Falotico et al., 1999). Under these management conditions, the soil NO_3^-–N concentration at sowing is generally low and relatively constant. Therefore, the predictive potential of models based solely on the NO_3^-–N content at sowing could be questionable because variable environmental conditions (soil temperature and moisture) and a short fallow period affect soil N mineralization processes. In general terms, only 38 to 54% of spring wheat yield variation would be explained by NO_3^-–N availability (0–60 cm) at sowing (Campbell et al., 1993; Barbieri et al., 2009).

The use of a complementary tool to quantify and estimate the N supply through mineralization throughout a crop

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Abbreviations: GNE, grain nitrogen export; GY_{0N}, grain yield of treatment without nitrogen; Nan, anaerobically incubated N; N_0, potentially mineralizable N; NT, no-till; SOM, soil organic matter.

Fig. 1. Southeastern Buenos Aires Province in Argentina (shaded area), with dots indicating the experimental sites.

cycle would improve the estimation of N availability and therefore the rate of N fertilizer to be applied. This would also increase N use efficiency, a relevant aspect from both economic and environmental points of view. Different biological and chemical tests have been proposed as indicators of soil N mineralization capacity (Griffin, 2008; Schomberg et al., 2009). Some researchers have suggested that NH_4^+–N produced during a 7-d anaerobic incubation (Nan) is the best biological indicator of potentially mineralizable N (Bushong et al., 2007; Soon et al., 2007). For conditions in southeastern Buenos Aires, Echeverría et al. (2000) reported a high correlation between Nan and potentially mineralizable N (N_0)

Table 1. Soil classification, preceding crop, years of continuous cropping (AA), soil organic matter (SOM), soil pH, anaerobically incubated N (Nan), NO_3^-–N, and rainfall between July and December (Pp) at the different experimental sites.

Year	Site	Soil (USDA)†	Preceding crop	AA	SOM	pH	Nan	NO_3^-–N (0–60 cm)	Pp
				yr	g kg^{-1}		mg kg^{-1}	kg ha^{-1}	mm
2006	Balcarce 1	PP	maize	15	50	5.8	58	39	314
	Balcarce 2	PP	sunflower	20	45	5.7	45	40	327
2008	Balcarce	PP	sunflower	15	59	6.2	48	75	330
	Otamendi 1	TA	soybean	15	61	5.8	48	59	330
	Otamendi 2	PP	soybean	20	60	6.1	48	54	310
2009	G. Madariaga 1	TA	soybean	2	68	5.6	93	120	410
	G. Madariaga 2	TA	maize	6	64	5.8	74	130	410
	Maipú	TA	soybean	20	48	5.7	54	117	380
	Pieres 1	TA	soybean	8	55	6.4	60	59	303
	Pieres 2	TA	soybean	15	53	5.8	60	59	320
	Miramar 1	TA	sunflower	10	59	5.9	51	91	317
	Miramar 2	TA	sunflower	10	60	6.0	60	93	317
	Miramar 3	PP	soybean	7	64	5.9	68	130	325
	Lobería	TA	sunflower	4	59	6.2	68	59	344
	Balcarce 1	TA	soybean	12	58	5.9	69	61	352
	Balcarce 2	PP	sunflower	5	50	6.5	74	46	352
2010	Miramar 1	TA	soybean	12	55	5.9	50	46	491
	Miramar 2	TA	soybean	12	44	5.7	50	68	480
	Pieres	TA	soybean	15	54	5.8	56	68	428
	Maipú 1	TA	soybean	7	59	5.9	73	43	445
	Maipú 2	TA	sunflower	9	49	5.7	61	58	445
	G. Madariaga	TA	maize	5	66	5.8	73	84	360
	Lobería	TA	sunflower	2	50	6.8	74	84	433
	Balcarce	TA	soybean	15	50	6.1	54	62	412
2011	Miramar	TA	soybean	10	65	5.6	58	81	450
	G. Madariaga	TA	soybean	6	65	5.8	77	74	355
	Maipú	TA	sunflower	6	56	5.9	73	78	350
	Lobería	TA	soybean	2	50	6.3	94	47	450
Avg.				10	56	6.0	62	74	377
SD				5.5	6.7	0.3	14	27.4	54.8

† TA, Typic Argiudoll; PP, Petrocalcic Paleudoll.

estimated through long-term aerobic incubations (224 d). Similar results were observed by Schomberg et al. (2009) in soils located in the southern United States. Moreover, Nan is a relatively simple indicator, sensitive to changes produced by management practices and tillage systems (Fabrizzi et al., 2003; Soon et al., 2007; Genovese et al., 2009; Cozzoli et al., 2010). Likewise, Sainz Rozas et al. (2008) reported that the use of Nan, along with NO_3^-–N, determined at planting or at the six-leaf stage, improved N availability diagnosis for corn. At present, however, there is no information about the usefulness of a combined index (NO_3^-–N + Nan) as an alternative to the use of a simple index (based only on NO_3^-–N) to improve N availability diagnosis for spring wheat. Thus the aim of this research was to assess the Nan contribution to explaining and predicting spring wheat yield, GNE, and the response to N fertilization.

MATERIALS AND METHODS

A total of 28 experiments were conducted under NT in 2006, 2008, 2009, 2010, and 2011 in fields with different farming histories in southeastern Buenos Aires Province, Argentina (from 34°41′ S, 58°27′ W to 38°23′ S, 58°40′ W). This area has an average annual temperature of 13.8°C and an average rainfall of 870 mm, 45% of which occurs during the wheat growing season. Early-season rain (June–September) is lower than potential evapotranspiration in three out of 30 yr. Late-season rain (October–December) is lower than potential evapotranspiration in 26 out of 30 yr (Calviño and Sadras, 2002). The experimental sites were located in Maipú, General Madariaga, Balcarce, Miramar, Otamendi, Pieres, and Lobería counties (Fig. 1). Predominant soils types were Typic Argiudolls and Petrocalcic Paleudolls, with loam surface texture, clay loam texture in the underlying horizon, and sandy loam texture below the 110-cm depth (C horizon). A Petrocalcic Paleudoll presents discontinuous layers of petrocalcic horizon below 0.8 m and greater clay contents in subsurface layers than Typic Argiudolls. Table 1 shows some soil characteristics of the experimental sites.

The experimental design was a randomized complete block with three replications and two treatment levels: (i) control without N fertilization and (ii) N rate for the maximum yield response. The application rates of N on the experimental sites ranged between 90 and 200 kg N ha^{-1} (Table 2). For P and S not to be limiting, 25 kg P ha^{-1} and 20 kg S ha^{-1} were applied at sowing. The N fertilizer was surface-broadcast urea (46% N) at the two- or three-leaf stage. The experimental unit size was 30 m^2 (3 m wide by 10 m long). Weeds, pests, and fungal diseases were controlled using appropriate pesticides at recommended rates.

Table 2. Yield and grain protein content for treatments with and without N at different experimental sites and N rate for the maximum yield response (NR).

Year	Site	NR	Grain yield		Protein	
			Without N (GY_{0N})	With N	Without N	With N
			kg ha^{-1}		g kg^{-1}	
2006	Balcarce 1	120	3700 b†	5228 a	98 b	125 a
	Balcarce 2	120	3198 b	4262 a	133 b	156 a
2008	Balcarce	100	4358 b	5041 a	94 a	99 a
	Otamendi 1	110	4526 b	5692 a	90 b	132 a
	Otamendi 2	90	4108 a	4409 a	106 b	131 a
2009	G. Madariaga 1	160	6942 b	8097 a	87 b	106 a
	G. Madariaga 2	160	5743 b	6767 a	99 a	106 a
	Maipú	120	4827 b	5490 a	85 a	92 a
	Pieres 1	120	5134 b	6340 a	99 b	119 a
	Pieres 2	140	5603 b	7625 a	85 b	99 a
	Miramar 1	100	4828 b	6100 a	95 b	107 a
	Miramar 2	100	4587 b	4923 a	86 b	106 a
	Miramar 3	100	5620 b	6147 a	91 b	99 a
	Lobería	200	4370 b	5847 a	95 b	124 a
	Balcarce 1	100	4700 b	5546 a	109 b	137 a
	Balcarce 2	100	3711 a	4191 a	115 b	134 a
2010	Miramar 1	150	3232 b	5710 a	86 a	87 a
	Miramar 2	150	5309 b	7033 a	85 b	93 a
	Pieres	150	5691 b	8386 a	87 b	101 a
	Maipú 1	160	5587 b	6331 a	95 b	105 a
	Maipú 2	150	3936 b	4459 a	97 a	106 a
	G. Madariaga	150	4951 b	6490 a	89 a	93 a
	Lobería	100	6808 b	7845 a	98 b	106 a
	Balcarce	150	4189 b	6437 a	89 b	97 a
2011	Miramar	150	5174 b	7803 a	86 b	94 a
	G. Madariaga	100	6585 b	7412 a	93 a	99 a
	Maipú	100	6205 b	6741 a	89 b	98 a
	Lobería	150	6887 b	9329 a	91 a	99 a
Avg.			5025	6274	95	109
SD			1074	1330	11	17

†In each row, means followed by the same letter are not significantly different according to LSD test at 5% probability.

Soil samples were taken at spring wheat sowing at the 0- to 20-, 20- to 40-, and 40- to 60-cm depths. For surface samples (0–20 cm), SOM content, pH, Nan, and NO_3^-–N were determined. For subsurface samples (20–40 and 40–60 cm), only NO_3^-–N was determined. Soil NO_3^-–N was extracted with KCl and determined by colorimetry using a UV-VIS spectrophotometer (Keeney and Nelson, 1982). To determine N availability (kg ha^{-1}) in the first 60-cm depth of soil, an average bulk density of 1.2 Mg m^{-3} was assumed (Fabrizzi et al., 2005). Soil organic matter was determined by the method of Walkley and Black (1934), and pH was measured with an electrode in a 1:2.5 (soil/water) suspension. Anaerobically incubated N was obtained by soil incubation under anaerobic conditions for 7 d at 40°C, and the NH_4^+–N produced was determined by steam microdistillation (Bremner and Keeney, 1965) as proposed by Gianello and Bremner (1986).

At maturity, nine 1-m-long crop rows (1.8 m^2) were chosen at random from each experimental unit and hand harvested. Ears were threshed with a stationary thresher, and grain moisture content was determined to express grain yield at 14% moisture. The total grain N concentration was determined by dry combustion (Dumas method) (Jung et al., 2003), with a Leco TruSpec CNS (Leco Corporation, 2008). Grain N concentration was transformed to protein using a factor of 5.7 (Rhee, 2001). Grain N was determined as the product of wheat yield at 0% grain moisture and N concentration in the grain.

Treatment effects (N rate) were evaluated using analysis of variance (PROC ANOVA) and regression analyses (PROC REG) (SAS Institute, 1988). Least significant difference (LSD) at the 0.05 level was calculated when the *F* statistic for treatments was significant. The stepwise selection method was used to determine the best variable combination to explain GY_{0N}, GNE, and response to N fertilization.

To explain the relationship between years under cultivation and Nan, the model proposed by Bartholomew and Kirkham (1960) was used:

$$V_t = V_e - (V_e - V_0)\exp(-rt) \qquad [1]$$

where V_t is the analyzed variable value (Nan, in mg kg^{-1}) at time *t*, V_e is the variable value at equilibrium, V_0 is the initial variable value, *r* is the exponential variation rate (yr^{-1}), and *t* is time since the beginning of cultivation (yr). Curve fitting was performed with PROC NLIN (SAS Institute, 1988).

Regression analysis was applied to a set of simulated and measured data comparisons to provide the coefficients of the estimated intercept, the coefficients of the estimated slope, and the R^2 value. Such an approach has been recommended (Hunt and Boote, 1998) for validation of model results. The equality of the intercept and of the slope to zero and one, respectively, was tested through *F* tests both separately and simultaneously. All calculations and analyses for model performance evaluation were done in the computational environment R (R Development Core Team, 2009).

RESULTS AND DISCUSSION

Observed pH values were among the range for soils typical of the region under long-term cropping (Sainz Rozas et al., 2011) (Table 1). The SOM content varied between 44 and 68 g kg^{-1}, while Nan concentrations and NO_3^-–N availability ranged between 45 and 94 mg N kg^{-1} and between 39 and 130 kg N ha^{-1}, respectively (Table 1). These variations in SOM, Nan, and NO_3^-–N could be attributed mainly to the effect of different lengths of time under cropping and different soil management practices because the surface soil texture was relatively similar in all sites (data not shown). Sainz Rozas et al. (2011) reported SOM contents averaging 88 and 55 g kg^{-1} in the surface layer of pristine soils and soils under continuous cropping, respectively. In addition, several researchers have reported changes in SOM or its more labile fractions due to different soil management practices (Studdert and Echeverría, 2000; Fabrizzi et al., 2003; Eiza et al., 2005), which helps to explain our results. The higher levels of NO_3^-–N determined at spring wheat sowing for most of the sites sampled in 2009 could be partially explained by the scarce rainfall during the preceding summer growing season (2008–2009) (between 260 and 380 mm), which resulted in lower yields and plant N uptake (Rimski-Korsakov et al., 2009).

Anaerobically incubated N concentrations were within the values reported by other researchers (Echeverría et al., 2000; Cozzoli et al., 2010; Reussi Calvo et al., 2011). Urquieta (2008) determined the average Nan content in pristine soils to be 138.4 mg kg^{-1}, with maximum and minimum values of 222 and 70.7 mg kg^{-1}, respectively. Therefore, the wide range of Nan values determined in this research represented conditions with different N mineralization potential.

The relationship between Nan concentration and SOM content is shown in Fig. 2a. No association was observed between those variables (R^2 = 0.14), which can be explained

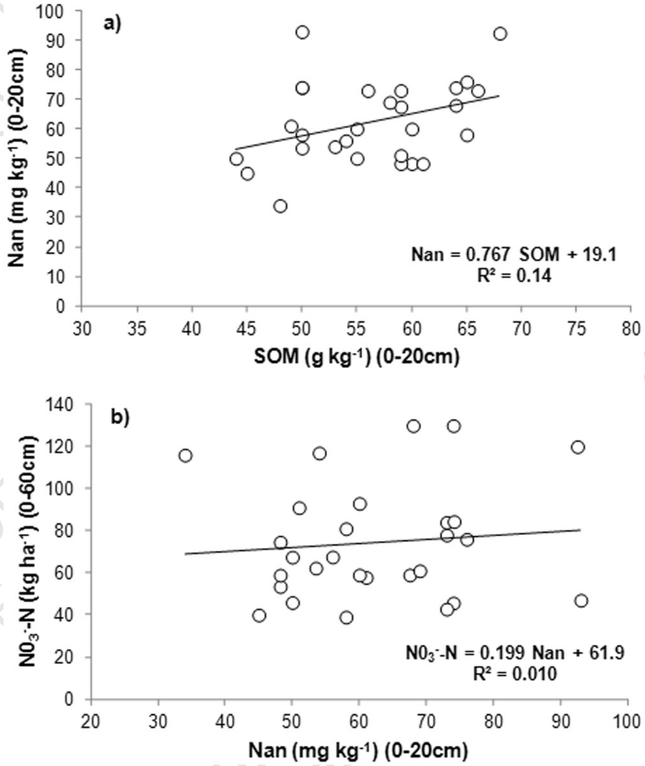

Fig. 2. (a) Ammonium-N produced in 7-d anaerobic incubations (Nan) as a function of soil organic matter (SOM) content, and (b) soil NO_3^-–N content at sowing as a function of Nan. Each circle represents the average of three replicates.

by the higher concentration of mineral-associated organic C (AOC) in relation to particulate organic C (POC) in fine textured soils (Cozzoli et al., 2010; Divito et al., 2011), like those used in this research. For this region, Studdert et al. (2006) determined a better relationship between Nan and POC than the relationship of Nan with soil SOM or AOC. Sharifi et al. (2008) observed a lower concentration of N_0 in soils with high clay content, while Van Veen and Kuikman (1990) determined a higher AOC accumulation in those soils than POC. These results differ from those reported by Springob and Kirchmann (2003), who reported a strong relationship between N_0 and total organic C (TOC) in sandy soils, which could be explained by a higher proportion of POC in TOC.

No significant relationship was found between Nan content and NO_3^-–N availability at sowing (Fig. 2b). This could be because soil NO_3^-–N content depends not only on Nan but also on the net balance between production (mineralization) and loss (leaching, denitrification, and immobilization) processes, which in turn depend on weather conditions of each particular year and on soil management practices (tillage system, preceding crop, etc.) (Genovese et al., 2009; Divito et al., 2011).

There was a close association ($P < 0.05$) between Nan concentration and years under continuous cropping (Fig. 3). For the southeastern Buenos Aires Province, Diovisalvi et al. (2009) reported that 85% of the fields under continuous cropping had Nan values <100 mg kg^{-1}, whereas only 35% of the fields under crop–pasture rotations had Nan below that value. Others researchers also observed that Nan concentration decreased with increasing years under continuous cropping (Urquieta, 2008; Genovese et al., 2009). Likewise, Cozzoli et al. (2010) determined higher levels of Nan under pasture than cropping under both NT and conventional tillage. This confirms the effect of soil management on Nan. Genovese et al. (2009), however, determined a greater decrease in Nan values and greater V_e (Eq. [1]) than those observed in our experiment (Fig. 3).

Spring wheat yield and grain protein content for the different N fertilization treatments and experimental sites are shows in Table 2. The average GY_{0N} was 3450, 4330, 5020, 5288, and 6262 kg ha^{-1} and the average response to N was 1296, 717, 1001, 1624, and 1559 kg ha^{-1} for 2006, 2008, 2009, 2010, and 2011, respectively. In general terms, the average water availability during the crop cycle was higher in 2010 and 2011 than the rest of the years (Table 1), which could partially explain yield variations across years. Furthermore, within each year, yield variability depends on soil water availability at sowing, N availability, and soil type (Calviño and Sadras, 2002; Sadras, 2004). On the contrary, the average grain protein content was 116, 97, 95, 91, and 90 g kg^{-1}, with an average response to N of 25, 24, 17, 8, and 8 g kg^{-1} for 2006, 2008, 2009, 2010, and 2011, respectively (Table 2). Inverse relationships between spring wheat yield and grain protein content have been observed by other researchers (Fowler, 2003), a situation generally present in production systems of the Pampa region.

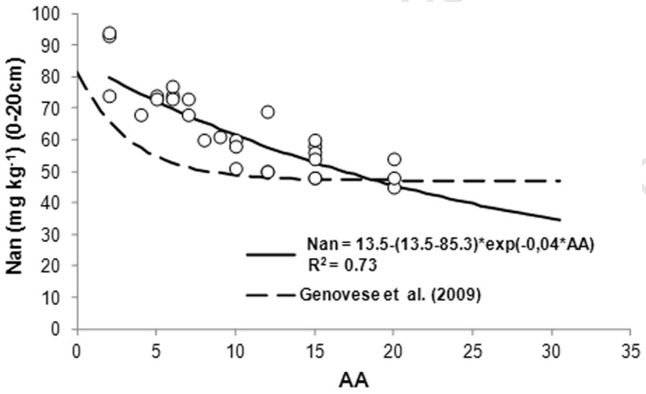

Fig. 3. Ammonium-N produced in 7-d anaerobic incubations (Nan) as a function of time under continuous cropping (AA, yr). Each dot represents the average of three replicates; the dashed line represents the model fitted by Genovese et al. (2009) [Nan = 47.1 – (47.1 – 81.4) exp(–0.3 AA)].

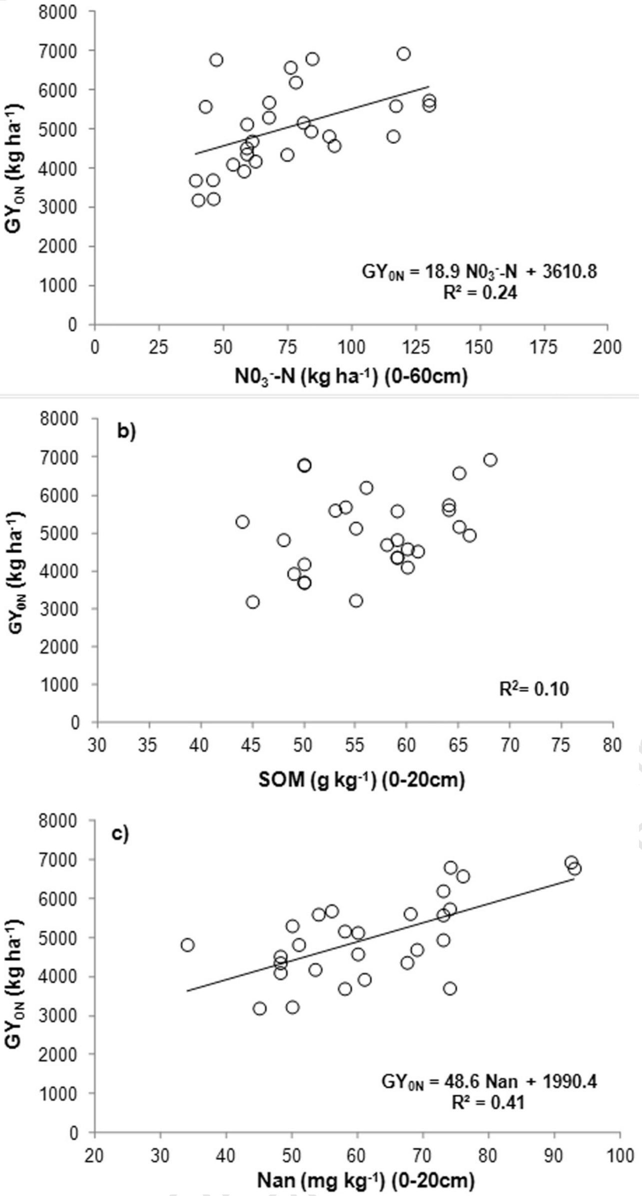

Fig. 4. Grain yield without N fertilizer (GY_{0N}) as a function of (a) NO_3^-–N availability, (b) soil organic matter (SOM) and (c) NH_4^+–N produced in 7-d anaerobic incubations (Nan). Each dot represents the average of three replicates.

Table 3. Models and model parameters for different soil variables.

Model	Dependent variable†	Variable‡	Parameter value	*P* value	Confidence limits (95%)		RMSE	Partial R^2	R^2
1	GY_{0N} (kg ha^{-1})	Intercept	3609		2537	4681	938		0.24
		NO_3^-–N	18.8	0.008	5.30	32.4		0.24	
2	GY_{0N} (kg ha^{-1})	Intercept	–1555		–4285	1178	652		0.66
		NO_3^-–N	80.7	0.009	19.3	142.1		0.18	
		NO_3^-–N^2	–0.38	0.031	–0.72	–0.02		0.07	
		Nan	47.4	0.001	28.7	66.1		0.41	
3	GNE (kg ha^{-1})	Intercept	57.8		43.0	72.6	12.9		0.11
		NO_3^-–N	0.17	0.070	–0.02	0.35		0.11	
4	GNE (kg ha^{-1})	Intercept	19.1		0.77	37.4	9.10		0.58
		NO_3^-–N	0.13	0.040	0.002	0.26		0.07	
		Nan	0.66	0.001	0.40	0.92		0.51	
5	response to N (kg ha^{-1})	Intercept	–625.7		–2606	1355	448.6		0.58
		Pp	7.20	0.002	3.00	11.4		0.35	
		Nan	–31.6	0.010	–54.9	–8.36		0.12	
		GY_{0N}	0.28	0.050	–0.014	0.58		0.11	

† GY_{0N}, grain yield of treatment without N; GNE, N in grain.

‡ NO_3^-–N (kg ha^{-1}, 0–60 cm); Nan, anaerobically incubated N (mg kg^{-1}, 0–20 cm); Pp, total precipitation from July to December.

Several researchers had reported that NO_3^-–N content at sowing is an acceptable indicator of N availability for crops (Calviño et al., 2002; Barbieri et al., 2009, 2012). Today, however, the generalized adoption of NT and the increase of high-yielding soybean as the preceding crop for spring wheat create conditions for low N availability at sowing (Echeverría et al., 1992; Studdert et al., 2000). Likewise, despite in these experiments N availability down to the 60-cm depth that varied from 39 to 130 kg N ha^{-1}, NO_3^-–N content only explained 24% of the GY_{0N} variability, which suggests the limitation of using this single-variable N availability diagnosis for spring wheat (Fig. 4a; Table 3, Model 1).

Some sites had high grain yields despite low NO_3^-–N concentrations, which suggests an important contribution of the mineralizable N pool after sowing (Fig. 4a). The soil SOM content did not help to explain ($P > 0.05$) GY_{0N} prediction (Fig. 4b), however, where a low relationship between Nan and SOM existed (Fig. 2a). Other researchers have also reported a weak association between SOM content and response to N by different crops (Álvarez and Steinbach, 2006; Quiroga et al., 2006). On the other hand, Nan concentration explained 41% of GY_{0N} variability (Fig. 4c). Domínguez et al. (2006) reported close associations between corn yield and Nan concentration under both NT and conventional tillage; however, Sainz Rozas et al. (2008) found only a weak association between those variables ($R^2 = 0.25$) for corn.

When both variables (NO_3^-–N availability and Nan) were taken into account in this experiment, the predictive capacity of GY_{0N} improved significantly ($R^2 = 0.66$) (Table 3, Model 2). Model 2 explained 66% of GY_{0N} variation, with a significant ($P < 0.05$) Nan contribution of 41%. It is worth mentioning, however, that grain yield can vary among sites due to soil type and the preceding crop (Echeverría et al., 1992) and among years due to rainfall (Calviño and Sadras, 2002). Thus, these results indicate that the initial NO_3^-–N content along with an estimation of the contribution by mineralization (Nan) should be considered in combination to quantify crop N availability with better accuracy. For example, considering the average values of NO_3^-–N availability and Nan (Table 1) and using Model 2 (Table 3), a yield of 5274 kg ha^{-1} GY_{0N} was predicted compared with an average GY_{0N} for the 28 sites of 5025 kg ha^{-1} (Table 1); the model overestimated yield by only 5%.

There was no significant contribution ($P < 0.05$) of SOM to GNE prediction when it was included in the model (data not shown), whereas NO_3^-–N content alone explained only 11% for this variable (Table 3, Model 3). When Nan concentration was incorporated into the model, however, its predictive capacity increased up to 58% (Models 3 and 4, Table 3). The partial contribution of Nan to GNE prediction was higher than its contribution to wheat GY_{0N} (51 and 41%, respectively; Table 3). Urquieta (2008) noted that the incorporation of Nan concentration together with NO_3^-–N in the model helps to explain variations in spring wheat protein content. Soil mineralization processes depend on many factors, including soil water content and temperature (Echeverría et al., 1994). As crop growth evolves, N mineralization increases (Studdert et al., 2000), justifying our results. Furthermore, Bashir et al. (1997) reported that N absorption during the postanthesis stage may contribute to GNE.

Information in Model 4 (Table 3) could be used to estimate GNE, and later, integrating this information with that generated with Model 2, protein concentration in samples [protein in g kg^{-1} = GNE × 1000 × 5.7/GY_{0N} (dry)] could be estimated. As an example, using NO_3^-–N availability and average Nan from the 28 sites (Table 1) it was possible to estimate a GNE of 69.6 kg ha^{-1} by using Model 4. Considering the average GY_{0N} estimated with Model 2 and corrected to 0 g kg^{-1} grain moisture content (4535.6 kg ha^{-1}), the average protein content would be 87.5 g kg^{-1}. Comparing this value with the average protein content (Table 2), it can be observed that the integration of the two models underestimated the GNE by only 7%.

Finally, a model emerged that enables N estimation based on Nan content, total rainfall during the crop cycle, and GY_{0N} estimation from Model 2 (Table 3, Model 5). Three sites were not included due to water stress (Balcarce in 2008, Otamendi 2 in 2008, and Miramar 3 in 2009) and two sites (Maipú in 2009 and Maipú 2 in 2010) due to viral disease (wheat streak mosaic virus). Sadras (2004) reported that in many field situations, colimitation by N and water stress exists. Water

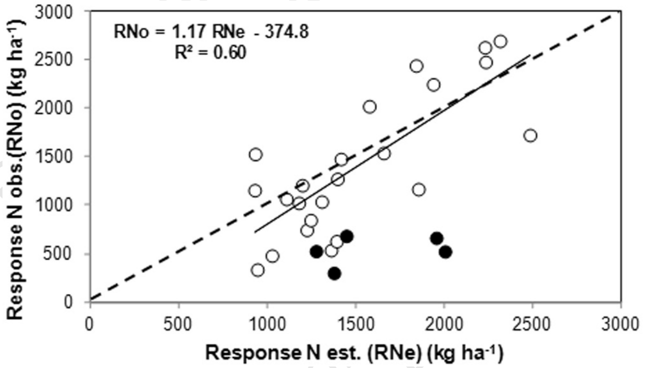

Fig. 5. Simple linear regression between observed (obs.) value of response to N (RN) and the estimated (est.) value obtained with Model 5 (Table 3). The filled symbols correspond to sites not included in the regression due to water and disease stress.

stress has a greater impact on yield in situations of high N availability, limiting crop response to added N. This would also help explain the lack of a significant contribution ($P < 0.05$) of total precipitation from July to December to GY_{0N} prediction when included in Model 2. The model parameter associated with Nan in Model 5 (Table 3) was negative, which indicates a lower response to N if Nan was higher (Sainz Rozas et al., 2008). On the contrary, coefficients for rainfall and GY_{0N} were positive, indicating that years with higher rainfall and with high GY_{0N} will result in a higher response to N (Calviño and Sadras, 2002). Therefore, using the information in Model 5 (Table 3), a requirement of 30 kg N Mg^{-1} (Calviño et al., 2002; Barbieri et al., 2012) and 50 to 60% efficiency of N recovery in the plant (Melaj et al., 2003; Barbieri et al., 2008; Velasco et al., 2012), it is possible to estimate the N rate for spring wheat with 58% reliability. For example, using Model 5 (Table 3), it is possible to estimate a response to N fertilization of 1987 kg ha^{-1} considering the average GY_{0N} and average rainfall of 430 mm (Barbieri et al., 2008). The estimated rate of N would be 99.4 kg N ha^{-1} (N rate = 1.987 Mg grain ha^{-1} × 50 kg N Mg^{-1}). There was a close association ($R^2 = 0.60$) between the observed and estimated N response with Model 5 (Fig. 5). The linear regression of observed N response vs. simulated values had intercepts not significantly different from 0 and slopes not significantly different from 1 ($P > 0.05$). The model predicted N response reasonably well, with a root mean squared variation value of 390 kg ha^{-1}.

In summary, Nan incorporation into traditional N availability diagnosis models for spring wheat improved GY_{0N} and GNE predictions (partial contribution $R^2 = 0.41$ and 0.51, respectively). With this information, the development of a model to predict response to N was possible, and therefore, N rate according to rainfall, GY_{0N}, and Nan; however, this model needs validation with an independent data set.

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