

# Diagnosis of sulfur availability for corn based on soil analysis

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**Abstract** Different edaphic properties were evaluated to diagnose soil sulfur (S) availability for corn in 15 field experiments as follows: (a) soil sulfate ( $\text{SO}_4^{-2}\text{-S}$ ) content at sowing at 0–20 cm and 0–60 cm depths [ $S_{\text{ini}(0-20)}$  and  $S_{\text{ini}(0-60)}$ ]; (b) soil  $\text{SO}_4^{-2}\text{-S}$  content at  $V_6$  corn stage at 0–20 and 0–60 cm depths [ $S_{V_6(0-20)}$  and  $S_{V_6(0-60)}$ ]; (c) potentially mineralizable S estimations [mineralizable S determined by short-term aerobic incubation ( $S_{\text{mineralized}}$ ), mineralizable N determined by short-term anaerobic incubation ( $N_{\text{an}}$ ), soil organic matter (SOM), SOM/clay ratio, and SOM/(clay + silt) ratio]; and (d) a combined index between  $S_{\text{ini}(0-60)}$  and potentially mineralizable S estimations. Three out of 15 sites presented grain yield response to S fertilization ( $p < 0.1$ ). The average yield response was  $1.06 \text{ Mg ha}^{-1}$  for these three sites. From the evaluated predictors,  $S_{\text{ini}(0-60)}$ ,  $S_{V_6(0-60)}$ , and  $N_{\text{an}}$  were the ones that better estimated the response to S fertilization, showing a linear-plateau relationship ( $R^2 = 0.68, 0.70, \text{ and } 0.62$ , respectively). Values greater than  $40 \text{ kg S ha}^{-1}$ ,  $59 \text{ kg S ha}^{-1}$ , and  $54 \text{ mg N kg}^{-1}$  for  $S_{\text{ini}(0-60)}$ ,  $S_{V_6(0-60)}$ , and  $N_{\text{an}}$ , respectively indicated no response to S fertilization. All other evaluated edaphic variables presented no relationship, or just a weak one, with S response. The incorporation of S mineralization indexes to the  $S_{\text{ini}(0-60)}$  model did not improve its performance. Our results indicate

that the evaluation of S mineralization has the potential to be used in S fertilization diagnoses.

**Keywords** Soil sulfate content · Moment and depth of sampling · Mineralizable S estimations · Incubations

## Introduction

Sulfur (S) deficiencies have been observed in the largest corn (*Zea mays* L.) producing countries as follows: the USA, China, Brazil, and Argentina (Hitsuda et al. 2008). The frequency and magnitude of these deficiencies have increased in recent years due to the limited use of S fertilizers, the intensification of agriculture, and the reduction of S concentration in the atmosphere (Scherer 2001). Additionally, the depletion of soil organic matter (SOM) (Durán et al. 2011; Sainz Rozas et al. 2011), which contains up to 95 % of the total S in the soil (Eriksen et al. 1998), has reduced S availability.

Mineral or organic fertilizers are often used to alleviate S deficiencies, but to achieve a rational use of these fertilizers, it is necessary to develop and calibrate S availability diagnostic methods (Blake-Kalff et al. 2002; Reussi Calvo et al. 2011; Bindraban et al. 2015; Divito et al. 2015). Soil sulfate concentration ( $\text{SO}_4^{-2}\text{-S}$ ) at sowing ( $S_{\text{ini}}$ ) has been broadly used to predict corn response to S fertilization (Rehm and Clapp 2008). Most of the studies have evaluated  $S_{\text{ini}}$  at a 0–20 cm depth ( $S_{\text{ini}(0-20)}$ ) with mixed results (Fox et al. 1964; Grobler et al. 1999; Van Biljon et al. 2004). This difference between studies is probably a consequence of the unaccounted variability in subsurface ( $>20 \text{ cm}$ )  $\text{SO}_4^{-2}\text{-S}$  concentration, which is very important for plant nutrition (Beaton and Soper 1986; San Martín and Echeverría 1995). This is why the determination of  $S_{\text{ini}}$  at 0–60 cm depth ( $S_{\text{ini}(0-60)}$ ) is necessary.

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Independently from the sampling depth,  $S_{ini}$  is the most traditional method to determine S availability for corn. Nevertheless, the predictive capacity of  $S_{ini(0-60)}$  was poor in most cases (Salvagiotti et al. 2005; Prystupa et al. 2006; Rehm and Clapp 2008; Sawyer et al. 2009; Pagani and Echeverría 2011). This low predictive capacity of  $S_{ini(0-60)}$  can be a consequence of the great spatial and temporal variability of soil  $SO_4^{-2}$  (Bloem et al. 2001), of the presence of shallow groundwater with high  $SO_4^{-2}$  concentration (Haneklaus et al. 2006), and of the unaccounted available S derived from mineralization during the growing season (Camberato et al. 2012). Therefore, it may be possible to improve corn S diagnosis by developing a combined index considering  $S_{ini}$  together with laboratory methods to estimate soil S mineralization potential.

There are no field calibrated methods to account for S mineralization on corn S diagnosis. For nitrogen (N), Magdoff et al. (1984) proposed to measure  $NO_3^-$ -N content at corn  $V_6$  stage (Ritchie and Hanway 1982). This methodology accounts for the initial soil  $NO_3^-$ -N content and for N mineralization from sowing to  $V_6$ , which can serve as an estimator of the soil N mineralization during the whole growing season (Magdoff 1991). Considering that N and S dynamics in soil are closely associated (Maynard et al. 1983; Echeverría et al. 1996), the quantification of  $SO_4^{-2}$ -S at  $V_6$  at a 0–20 or 0–60 cm depth ( $S_{V6(0-20)}$  and  $S_{V6(0-60)}$ , respectively) appears as a promising alternative to improve S response predictions, which has never been evaluated before.

Another strategy to account for mineralization in S diagnosis is the use of laboratory methods to estimate soil S mineralization potential. The potentially mineralizable S ( $S_0$ ) measured by long-term aerobic incubations (10 to 40 weeks) (Pirela and Tabatabai 1988; Ghani et al. 1991; Tanikawa et al. 2014) is often used as a standard. However, these incubations are laborious and lengthy, making them unsuitable as routine analysis in soil testing labs. An alternative to long-term incubations is the determination of the  $SO_4^{-2}$ -S released after one week of aerobic incubation ( $S_{mineralized}$ ). This short-term incubation is highly correlated with  $S_0$  (Wyngaard and Cabrera 2015) and is capable of discriminating soils with different S mineralization potentials (Carciochi et al. 2014). However,  $S_{mineralized}$  has never been evaluated as an index to estimate corn available S. Sulfur mineralization can be also potentially predicted by indexes to estimate N mineralization. Among these indexes, the quantification of  $NH_4^+$ -N after a 7-day anaerobic incubation ( $N_{an}$ ) (Waring and Bremner 1964) has been described as an efficient estimation of  $S_0$  (Wyngaard and Cabrera 2015).

The SOM is a buffer for  $SO_4^{-2}$ -S in soil solution (Ghani et al. 1991), and has been suggested as an index of S mineralization potential. Indeed Riffaldi et al. (2006) and Wyngaard and Cabrera (2015) observed a strong relationship between SOM and  $S_0$ , while opposite results were described by Tabatabai and Al-Khafaji (1980) and Pirela and Tabatabai (1988). A possible explanation for these contrasting results

is that the S mineralization rate does not only depend on the size of the organic pool, but also on edaphic properties such as soil texture (Tanikawa et al. 2014), because clay and silt particles protect SOM from decomposition (Six et al. 2002). Consequently, the use of combined indexes accounting for both SOM and soil texture [SOM/clay or SOM/(clay + silt)] may better predict S mineralization potential than just SOM.

Further research is required on strategies to account for S mineralization in S diagnostic methods. Along this line, the comparison between  $S_{ini}$  and  $S_{V6}$  would allow to evaluate the diagnostic capacity of the newly proposed  $S_{V6}$  method, while the comparison between  $S_{ini(0-20)}$  and  $S_{ini(0-60)}$  or  $S_{V6(0-20)}$  and  $S_{V6(0-60)}$  would allow to determine the best soil sampling depth for each method. Additionally, the use of S mineralization estimations [ $S_{mineralized}$ ,  $N_{an}$ , SOM, SOM/clay, and SOM/(clay + silt)] to diagnose S availability for corn, or their incorporation to the traditional diagnostic strategy based on  $S_{ini}$ , has never been evaluated before.

Our objectives were to evaluate and compare different methods to diagnose corn S availability based on the following indexes: (a)  $S_{ini(0-20)}$  and  $S_{ini(0-60)}$ , (b)  $S_{V6(0-20)}$  and  $S_{V6(0-60)}$ , (c) potentially mineralizable S estimations [ $S_{mineralized}$ ,  $N_{an}$ , SOM, SOM/clay, and SOM/(clay + silt)], and (d) a combined index including  $S_{ini(0-60)}$  and potentially mineralizable S estimations.

## Materials and methods

### Crop management, experimental sites, and design

Fifteen field experiments were carried out during the 2013/14 and 2014/15 seasons (Table 1). These experiments were located in the northern, north-central, south-central, and southern region of the Argentinean Pampas (Table 1), where corn is the main summer cereal crop. Trials were centered around Rafaela (31.2° S, 61.3° W) in the northern region, Villa Cañas (34.0° S, 61.4° W) in the north-central region, 9 de Julio (35.3° S, 60.5° W) in the south-central region, and Balcarce (37.5° S, 58.2° W) in the southern region. These geographical areas were selected to obtain a wide range of edaphic conditions and S availability levels. The experimental design was a randomized complete block arrangement with three replicates (plot size 12 × 5 m). Plant density ranged between 56,000 and 85,800 plants ha<sup>-1</sup>, depending on the site. Gypsum (CaSO<sub>4</sub> · 2H<sub>2</sub>O, 18 % S) was broadcast at crop emergence at 0, 8, 16, 24, and 32 kg S ha<sup>-1</sup> in 11 experiments and at 0, 16, and 32 kg S ha<sup>-1</sup> in four experiments. Nitrogen (200 kg N ha<sup>-1</sup>) and phosphorus (30 kg P ha<sup>-1</sup>) were applied to all plots as urea (46 % N) and triple superphosphate (20 % P) to avoid nutrient deficiencies. All experiments were performed under no tillage, without irrigation, and in soils with deep groundwater tables (below rooting zone).

**Table 1** Site description, crop management (planting date), soil characteristics (soil type, texture, silt and clay content, pH), and rainfall during the whole crop growing season (total) and during the critical period (CP) (15 days before and 15 days after silking)

Site description			Crop management	Soil characteristics					Rainfall	
Year	Site	Location	Planting date	Soil type (USDA)	Texture	Silt (g 100 g <sup>-1</sup> )	Clay (g 100 g <sup>-1</sup> )	pH	Total (mm)	CP (mm)
2013/14	S1	N-C	17 Oct	TH	Sandy loam	22.2	11.6	6.1	413	55
	S2	N-C	10 Dec	TH	Sandy loam	25.1	16.4	6.4	692	157
	S3	N	18 Dec	TA	Silt loam	63.2	18.3	6.0	676	177
	S4	N	19 Dec	TA	Silt loam	55.2	26.3	8.4	676	177
	S5	N	26 Dec	TA	Silt clay loam	62.2	27.0	5.9	662	215
	S6	N	18 Dec	TA	Silt clay loam	53.6	31.1	6.1	676	177
	S7	S	18 Oct	TA	Sandy loam	24.0	16.2	5.8	578	144
	S8	S	22 Oct	TA	Loam	33.6	19.4	5.9	594	144
	S9	S	22 Oct	TA	Loam	29.1	20.6	5.9	594	144
	S10	S	26 Oct	TA	Sandy clay loam	25.9	23.9	5.9	594	144
	S11	S	21 Nov	TA	Clay loam	34.5	28.3	6.4	556	138
2014/15	S12	S-C	20 Sept	EH	Sandy loam	11.9	16.8	5.9	576	73
	S13	S-C	19 Sept	EH	Sandy clay loam	25.6	22.5	5.8	576	73
	S14	S	7 Nov	TA	Sandy clay loam	21.2	26.7	6.1	367	110
	S15	S	26 Oct	TA	Clay loam	32.8	31.9	5.8	446	107

*N* north, *N-C* north-center, *S-C* south-center, *S* south, *TH* typic Hapludoll, *TA* typic Argiudoll, *EH* entic Hapludoll

Weeds were controlled by the application of glyphosate [N-(phosphonomethyl)glycine] at a 1.44 kg a.i. ha<sup>-1</sup> rate. When necessary, insects were controlled with chlorantraniliprole [5-bromo-N-(4-chloro-2-methyl-6-(methylcarbamoyl)phenyl)-2-(3-chloropyridin-2-yl)pyrazole-3-carboxamide] at a 1.6 g a.i. ha<sup>-1</sup> rate.

Rainfall data (Table 1) for the whole crop growing season and the critical period (CP) (15 days before and 15 days after silking) was obtained from research meteorological stations located in or near the experimental sites. Additional information on crop management and some soil characteristics are described in Table 1.

At physiological maturity (R<sub>6</sub>), ears from three crop rows (6 m long) were hand harvested from each experimental unit and threshed with a stationary thresher. Grain yield was expressed at 14 % moisture content.

### Soil sampling and laboratory procedure

At corn sowing, composite soil samples (14 subsamples per block) were taken at 0–20, 20–40, and 40–60 cm depths. Additionally, at V<sub>6</sub> stage soil samples were taken in nine out of 15 sites at 0–20, 20–40, and 40–60 cm depths from unfertilized plots. Samples were dried at 30 °C and ground to pass a 2-mm sieve for all analysis except for SOM, when a 0.5-mm sieve was used.

For surface soil samples (0–20 cm), pH, SOM (Walkley and Black 1934), and texture (Bouyoucos 1962) were determined. Soluble and adsorbed S as sulfate (SO<sub>4</sub><sup>2-</sup>-S) was determined at all depths by ion chromatography (IC) (Metrohm

IC 820 separation system, 819 conductivity detector with carbonate and cation suppression) after extraction of soil samples with 0.01 M NH<sub>4</sub>Cl at a 10:1 solution/soil ratio (Maynard et al. 1987). The bulk density of each site, estimated as proposed by Hollis et al. (2012), was used to convert SO<sub>4</sub><sup>2-</sup>-S concentrations from mg kg<sup>-1</sup> to kg ha<sup>-1</sup>. The SO<sub>4</sub><sup>2-</sup>-S determined at sowing (kg ha<sup>-1</sup>) was termed S<sub>ini(0–20)</sub> and S<sub>ini(0–60)</sub> (depending on sampling depth), and the SO<sub>4</sub><sup>2-</sup>-S determined at V<sub>6</sub> stage S<sub>V6(0–20)</sub> and S<sub>V6(0–60)</sub> (depending on sampling depth).

To determine S<sub>mineralized</sub> (in 0–20 cm depth), the technique proposed by Keeney and Bremner (1962) was performed. Ten g soil were thoroughly mixed with 30 g of acid-washed sand and transferred to a 50-mL plastic container. After this, the soil was moistened to 80 % of field capacity water content (Maynard et al. 1983), covered with a porous plastic film (PARAFILM®, Menasha, WI), and incubated at 40 °C for 7 days. Soil samples were weighed every 3 days to correct for water content. After the incubation period, SO<sub>4</sub><sup>2-</sup>-S was quantified as previously described and the initial SO<sub>4</sub><sup>2-</sup>-S concentration was subtracted from the final value. To determine N<sub>an</sub>, 10 g soil were saturated with distilled water and incubated at 40 °C for 7 days (Keeney 1982). The NH<sub>4</sub><sup>+</sup> produced during this period was quantified by steam micro-distillation (Bremner and Keeney 1965).

### Data analysis

Yield response in each site was analyzed using the ANOVA procedure included in the R software (R core team 2016).

Significantly different means were compared using a Tukey test at  $p=0.1$ . The relationship between variables was described with quadratic and linear-plateau models:  $y = a + b \times x$  if  $x \leq c$  and  $y = a + b \times c$  if  $x > c$ , where “ $a$ ” is the intercept, “ $b$ ” is the slope during the linear phase, and “ $c$ ” is the value of “ $x$ ” at which the linear model reaches a plateau.

In those sites-years where corn grain yield was not affected by S fertilization, yield response was calculated as the difference between the average yield of the fertilized treatments (8, 16, 24, and 32, or 16 and 32 kg S ha<sup>-1</sup>) and the yield of the S-unfertilized plot (0 kg S ha<sup>-1</sup>). When S fertilization effect on yield was significant, linear-plateau models between S rate and yield were fit to determine the maximum yield (plateau). The response of the crop to S fertilization was calculated as the yield difference between the control (0 kg S ha<sup>-1</sup>) and the plateau yield.

## Results and discussion

### Weather conditions

Total rainfall during the growing season ranged between 367 and 692 mm depending on the site and season (Table 1). In S1, S14, and S15 total rainfall was 413, 367, and 446 mm, respectively. These values were below corn water demand (approximately 500–600 mm). Also in these sites, water availability was limited around the critical period (Table 1), when grain number is defined (Andrade et al. 1993, 2002). Consequently, it is likely that yield has been negatively affected in these sites. However, in these sites, water availability was sufficient at crop emergence, when the S fertilizer was applied (data not shown). In all other sites and years, the amount and distribution of precipitations ensured sufficient water availability during most of the growing season. The average daily mean temperature and radiation were similar to the historical record for each region and season and did not negatively affect crop growth (data not shown).

### Grain yield

Average grain yields were 10.5, 13.3, 11.6, and 11.3 Mg ha<sup>-1</sup> for the northern, north-central, south-central, and southern areas, respectively (Table 2). Sulfur fertilization increased grain yield in three out of 15 sites (S1, S12, and S13) (Table 2). Average grain yield response to S fertilization in these three sites was 1.06 Mg ha<sup>-1</sup> (9.5 %) and it ranged from 0.95 to 1.2 Mg ha<sup>-1</sup>. This response was similar to that reported by other authors in the same region (Prystupa et al. 2006; Pagani et al. 2012) and in other countries as Nigeria and the USA (Kang and Osiname 1976; Fernández et al. 2012).

**Table 2** Corn grain yield at different S rates in 15 field experiments

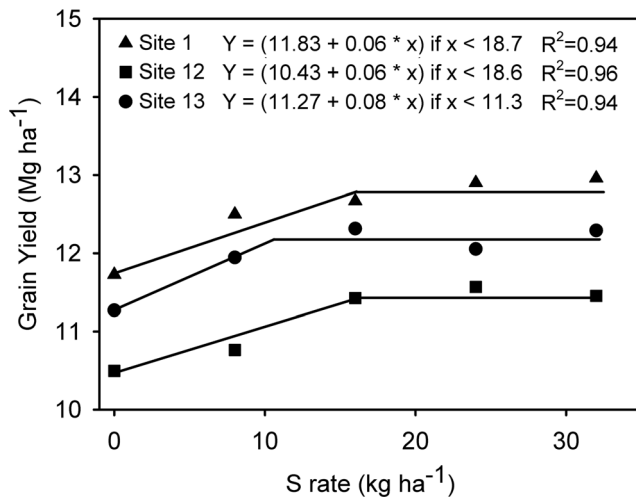
Site	S rate (kg S ha <sup>-1</sup> )				
	0	8	16	24	32
	Grain yield (Mg ha <sup>-1</sup> )				
S1	11.73 a	12.50 ab	12.67 ab	12.90 ab	12.96 b
S2	14.02 a	14.33 a	13.78 a	15.02 a	13.38 a
S3	9.03 a	9.79 a	9.63 a	9.85 a	9.49 a
S4	12.37 a	12.49 a	12.34 a	12.19 a	12.62 a
S5	9.45 a	9.46 a	9.80 a	9.79 a	10.42 a
S6	10.00 a	–	10.24 a	–	10.02 a
S7	9.21 a	–	9.73 a	–	9.02 a
S8	11.08 a	–	11.05 a	–	11.40 a
S9	10.54 a	–	10.40 a	–	11.11 a
S10	12.03 a	11.72 a	12.49 a	12.60 a	12.61 a
S11	11.42 a	12.02 a	11.42 a	11.90 a	11.72 a
S12	10.50 a	10.76 ab	11.43 b	11.57 b	11.45 b
S13	11.27 a	11.95 ab	12.32 ab	12.06 b	12.29 b
S14	8.55 a	8.99 a	9.06 a	9.12 a	8.82 a
S15	13.62 a	12.96 a	14.09 a	14.30 a	14.37 a
mean	10.99	11.54	11.36	11.94	11.45
SD	1.62	1.63	1.55	1.83	1.65

Different letters in the same line indicate differences between S rates at  $p < 0.1$  using Tukey test

The linear-plateau models between S rate and grain yield in S-responsive sites showed that maximum grain yield was reached at 18.7, 18.6, and 11.3 kg S ha<sup>-1</sup> in sites S1, S12, and S13, respectively (Fig. 1). Similar results were obtained in other experiments carried out in the same region (Pagani and Echeverría 2011), showing that S deficiencies in Argentina are not as strong as in other countries such as the USA, Pakistan, or India where maximum yield was reached at S rates ranging from 22 to 45 kg S ha<sup>-1</sup> (Rabuffetti and Kamprath 1977; Rasheed et al. 2004; Maurya et al. 2005; Jeet et al. 2012; Sutradhar and Fernandez 2015). This difference may be due to the lower SOM content (<20 g kg<sup>-1</sup>) and coarser texture in the soils where these last experiments were carried out.

### Soil analysis

Values for  $S_{ini}$  ranged from 9.7 to 21.8 kg ha<sup>-1</sup> (average 15.8 kg ha<sup>-1</sup>) in the 0–20 cm layer and from 22.3 to 61.3 kg ha<sup>-1</sup> (average 38.4 kg ha<sup>-1</sup>) in the 0–60 cm layer (Table 3). These values coincide with those previously reported in the studied area (San Martín and Echeverría 1995; Prystupa et al. 2006), and in other agricultural soils of the world (Fernández et al. 2012; Jeet et al. 2012; Sawyer et al. 2012). Additionally Table 3 shows that  $S_{ini(0-20)}$  represented 41 % of  $S_{ini(0-60)}$  demonstrating a slightly higher



**Fig. 1** Relationship between grain yield and S rate in the three sites with response to S (S1, S12, and S13)

concentration of S in the top soil layer. Average  $S_{V6(0-20)}$  and  $S_{V6(0-60)}$  values were 20.3 and 44.4  $kg\ ha^{-1}$ , which are 30.7 and 28.3 % greater than  $S_{ini(0-20)}$  and  $S_{ini(0-60)}$ , respectively. This is a consequence of the  $SO_4^{2-}$ -S released by mineralization between sowing and  $V_6$  stage, which is accounted for by  $S_{V6}$  but not by  $S_{ini}$ . Similar trends were reported for  $NO_3^-$ -N between these two sampling dates (Sarrantonio and Scott 1988; Sainz Rozas et al. 2008).

Among the methods to estimate S mineralization,  $S_{mineralized}$  ranged from 0.6 to 3.2  $mg\ S\ kg^{-1}$ , while  $N_{an}$  ranged from 19.3 to 136.5  $mg\ N\ kg^{-1}$  (Table 3). These values are within those reported in the same area by Carciochi et al. (2014) for  $S_{mineralized}$  and Sainz Rozas et al. (2008) and Reussi Calvo et al. (2013) for  $N_{an}$ . Moreover, Wyngaard and Cabrera (2015) reported similar  $S_{mineralized}$  and  $N_{an}$  values in contrasting soils from the United States. The broad range of values in our study is likely explained by differences in texture and SOM among sites. The SOM content varied from 20.8 to 73.5  $g\ kg^{-1}$ , and it was greater in the southern sites (59.4  $g\ kg^{-1}$ ) as compared to all other regions (average 34.7  $g\ kg^{-1}$ ). These values are among the typical range for arable soils in the Argentinean Pampas (Sainz Rozas et al. 2011), but are greater than those described in other soils of the world presenting S deficiencies (Grobler et al. 1999; Rehm 2005). The SOM/clay ratio varied from 1.1 to 3.8, while SOM/(clay + silt) ranged from 0.3 to 1.6 (Table 3).

**Performance of  $S_{ini}$  and  $S_{V6}$  diagnostic methods**

We observed a linear-plateau relationship between corn yield response to S fertilization and each  $SO_4^{2-}$ -S contents ( $S_{ini(0-20)}$ ,  $S_{ini(0-60)}$ ,  $S_{V6(0-20)}$ , and  $S_{V6(0-60)}$ ) (Fig. 2). The threshold  $S_{ini(0-20)}$  value above which there was no response

**Table 3**  $SO_4^{2-}$ -S content in soil at sowing at 0–20 cm ( $S_{ini(0-20)}$ ) and 0–60 cm depth ( $S_{ini(0-60)}$ ) and at  $V_6$  stage at 0–20 cm depth ( $S_{V6(0-20)}$ ) and 0–60 cm depth ( $S_{V6(0-60)}$ ), mineralizable S determined by short-term

aerobic incubation ( $S_{mineralized}$ ), mineralizable N determined by short-term anaerobic incubation ( $N_{an}$ ), soil organic matter (SOM), SOM/clay ratio, and SOM/(clay + silt) ratio

Site	$S_{ini(0-20)}$	$S_{ini(0-60)}$	$S_{V6(0-20)}$	$S_{V6(0-60)}$	$S_{mineralized}$	$S_0$	$N_{an}$	SOM	SOM/clay	SOM/(clay + silt)
	$kg\ S\ ha^{-1}$				$mg\ S\ kg^{-1}$		$mg\ N\ kg^{-1}$	$g\ kg^{-1}$		
S1	11.22	23.29	nd	nd	0.68	3.98	36.68	30.73	2.65	0.91
S2	14.51	34.95	29.87	60.89	0.94	3.84	48.36	34.51	2.10	0.83
S3	12.30	33.54	nd	nd	0.57	4.03	38.49	25.80	1.41	0.32
S4	21.75	49.42	36.78	75.37	3.18	8.67	136.49	68.34	2.60	0.84
S5	15.14	38.61	25.49	47.17	1.16	3.50	41.07	29.00	1.07	0.32
S6	19.54	44.64	nd	nd	1.48	6.06	73.36	33.94	1.09	0.40
S7	20.44	41.62	nd	nd	1.69	7.64	54.39	62.15	3.84	1.55
S8	16.15	61.29	nd	nd	1.38	7.38	62.82	59.97	3.09	1.13
S9	18.30	59.63	nd	nd	0.92	8.11	83.58	73.50	3.57	1.48
S10	20.24	47.38	18.79	48.30	0.92	5.95	46.29	54.81	2.30	1.10
S11	18.79	34.47	15.68	43.49	1.33	5.10	60.88	59.15	2.09	0.94
S12	10.03	22.30	10.47	26.47	0.57	2.57	19.26	20.81	1.24	0.73
S13	13.96	27.40	12.60	30.53	0.86	3.43	39.28	34.03	1.51	0.71
S14	9.69	23.03	11.92	25.68	0.65	2.19	31.46	43.80	1.64	0.91
S15	15.50	34.10	20.92	41.79	0.62	6.51	63.02	62.38	1.96	0.96
Mean	15.84	38.38	20.28	44.41	1.13	5.26	55.69	46.20	2.14	0.88
SD	3.92	12.37	8.94	16.30	0.67	2.08	27.97	17.36	0.87	0.36

nd not determined

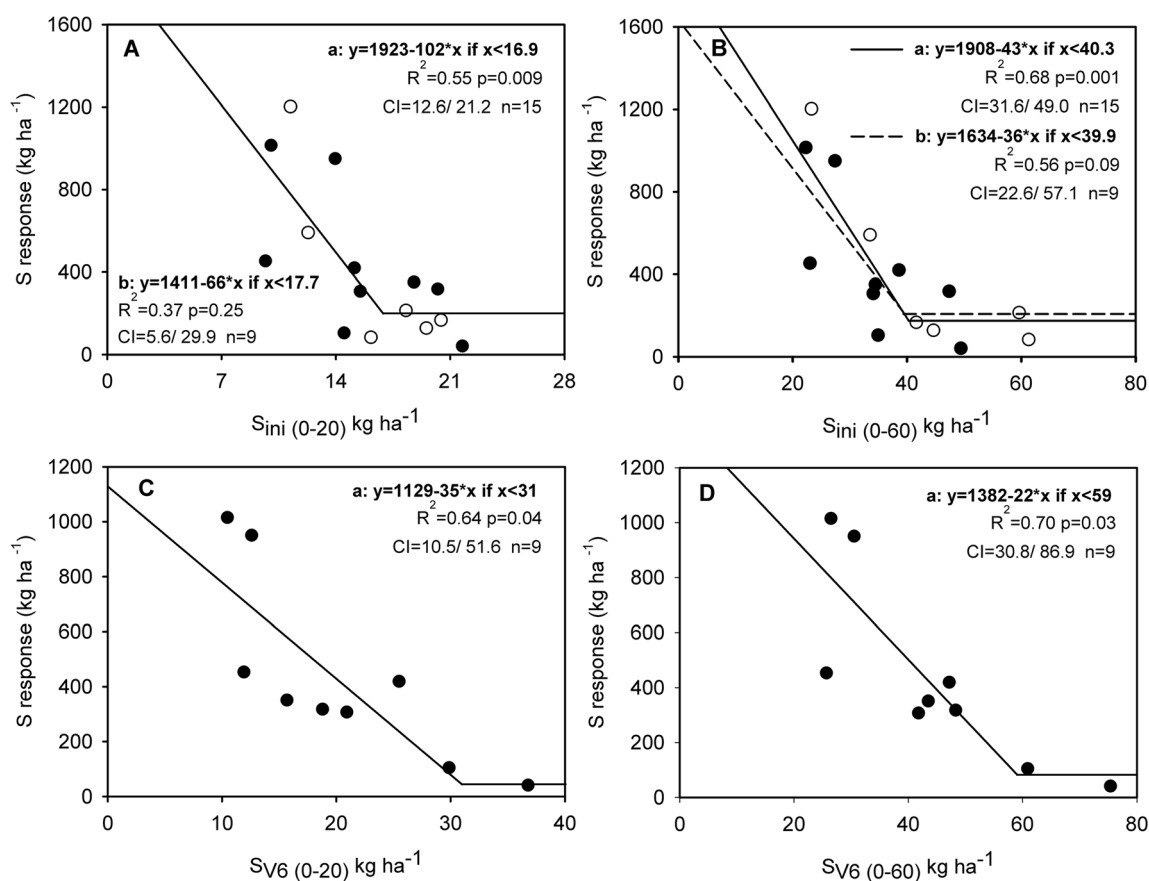
to fertilization was  $17 \text{ kg S ha}^{-1}$  (ca.  $7 \text{ mg kg}^{-1}$ ) (Fig. 2a). This value is similar to that reported by Fox et al. (1964) ( $8 \text{ mg kg}^{-1}$ ) but is slightly lower than that reported in other studies ( $10 \text{ mg kg}^{-1}$ ) (Grobler et al. 1999; Van Biljon et al. 2004). The confidence interval for this threshold ( $13$  to  $21 \text{ kg ha}^{-1}$ ) was in line with that described by Fox et al. (1964) ( $10$  to  $19 \text{ kg ha}^{-1}$ ), Kang and Osiname (1976) ( $10$  to  $20 \text{ kg ha}^{-1}$ ), and Fernández and Hoefl (2009) ( $14$  and  $25 \text{ kg ha}^{-1}$ ). This wide confidence interval suggests that  $S_{\text{ini}(0-20)}$  does not have a good performance for the diagnosis of S availability for corn.

For  $S_{\text{ini}(0-60)}$  the “c” value of the linear-plateau model was  $40 \text{ kg S ha}^{-1}$  (Fig. 2b), with a confidence interval between  $32$  and  $49 \text{ kg S ha}^{-1}$ . In all non-responsive sites the  $S_{\text{ini}(0-60)}$  was above this range, while three out of four sites with  $S_{\text{ini}(0-60)}$  values below  $32 \text{ kg S ha}^{-1}$  were responsive to S fertilization. The good performance of the  $S_{\text{ini}(0-60)}$  model to predict corn response to S fertilization contradicts previous reports (Prystupa et al. 2006; Rehm and Clapp 2008; Sawyer et al. 2009; Pagani and Echeverría 2011; Sawyer et al. 2012), but is

in line with Beaton and Soper (1986), who reported a threshold of  $36 \text{ kg S ha}^{-1}$  above which no response to S is expected.

The  $S_{\text{ini}(0-20)}$  explained only 55 % of corn response to S fertilization variability, while  $S_{\text{ini}(0-60)}$  explained 68 % of it (Fig. 2a and b). This highlights the importance of accounting for the  $\text{SO}_4^{2-}\text{-S}$  in the subsurface soil ( $>20 \text{ cm}$ ), which has been proved to be variable between sites and is very important for the crops S nutrition (Hoefl et al. 1985; Beaton and Soper 1986; Kamprath and Jones 1986; Fernández et al. 2012).

The  $S_{V6}$  explained corn response to S fertilization, being  $S_{V6(0-60)}$  a slightly better predictor of corn S response than  $S_{V6(0-20)}$  ( $R^2 = 0.64$  vs.  $0.70$ , Fig. 2c, d). The determination coefficient was also greater for the  $S_{V6}$  model as compared to  $S_{\text{ini}}$  at both depths (Fig. 2). This could be a consequence of  $S_{V6}$  partially taking into account the S mineralized during corn growing season. Similar results have been observed for N, as the quantification of soil  $\text{NO}_3^- \text{-N}$  in samples taken at  $V_6$  better predicted corn N response than those taken at sowing (Bundy et al. 1999; Sainz Rozas et al. 2008).



**Fig. 2** Linear-plateau models to describe the relationship between corn response to S fertilization and **a**  $\text{SO}_4^{2-}\text{-S}$  content in soil at sowing 0–20 cm depth ( $S_{\text{ini}(0-20)}$ ), **b** 0–60 cm depth ( $S_{\text{ini}(0-60)}$ ), **c**  $\text{SO}_4^{2-}\text{-S}$  content in soil at  $V_6$  stage 0–20 cm depth ( $S_{V6(0-20)}$ ), and **d** 0–60 cm depth ( $S_{V6(0-60)}$ ). Filled markers in **a** and **b** are the same nine sites where  $S_{V6}$  was

determined. Model “a” and full line in graphics **a** and **b** belong to the 15 sites evaluated while model “b” and dotted line belong to the same nine sites as  $S_{V6}$ . CI is the confidence interval of the critical threshold (0.95) and  $p$  indicates the significance of the regression

Even if  $S_{mi(0-60)}$  and  $S_{V6(0-60)}$  significantly predicted corn S response, some precautions should be taken when using these methods. First, soil samples should be taken from homogeneous and representative areas due to the great spatial variability of  $SO_4^{-2}$  as well as other edaphic properties (Bloem et al. 2001; Haneklaus et al. 2007). Secondly, heavy rainfall events after soil sampling can leach  $SO_4^{-2}$  from the soil profile and lead to an overestimation of S availability (Haneklaus et al. 2006). Finally, the presence of a groundwater table in the rooting zone, or the use of groundwater for irrigation, can reduce the predictive capacity of soil-based diagnostic methods (Bloem et al. 2000). This is because groundwater can act as a source of available S during the growing season.

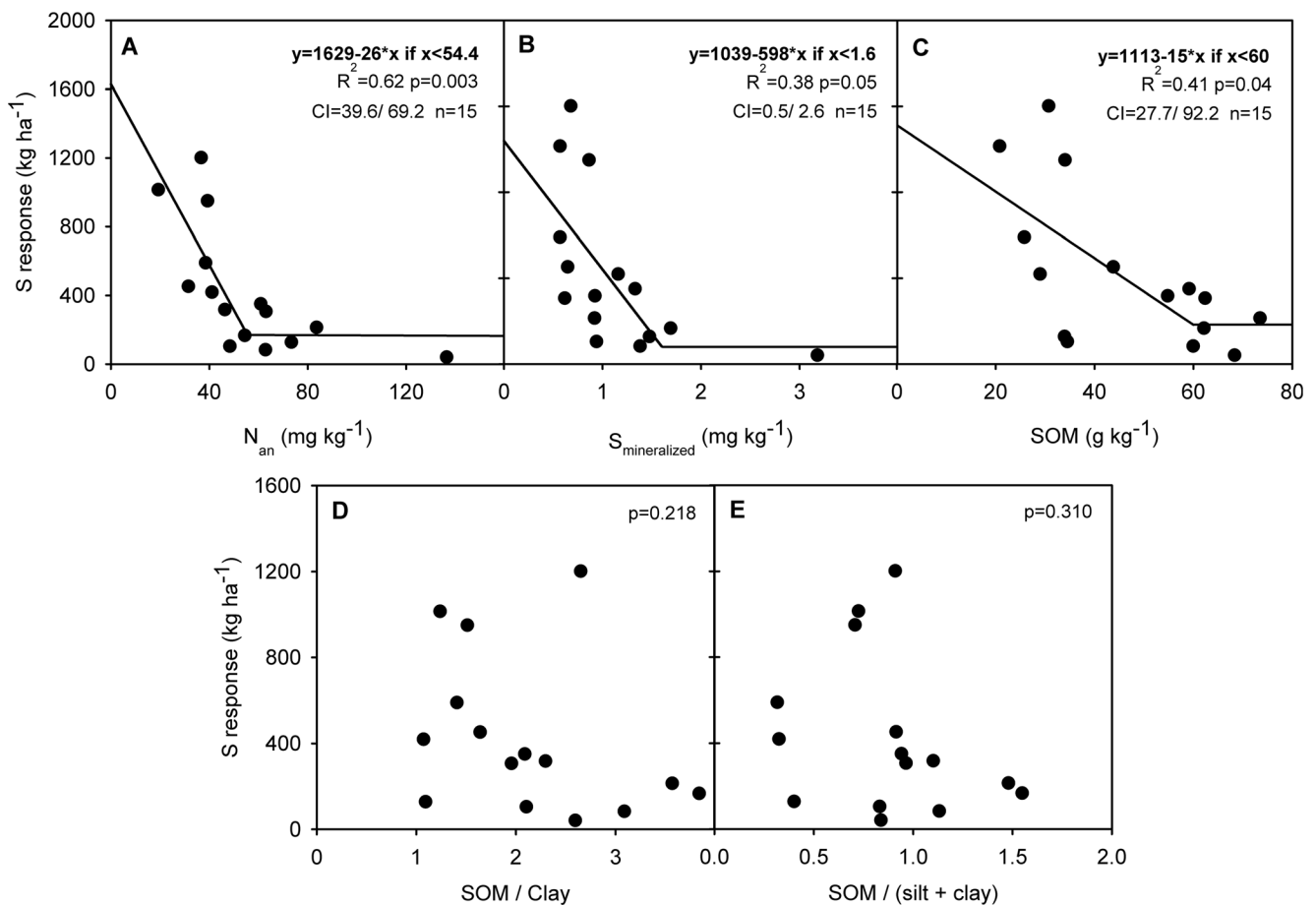
**Performance of S mineralization estimations**

We determined a significant linear-plateau relationship between  $N_{an}$ ,  $S_{mineralized}$ , or SOM and corn response to S fertilization (Fig. 3). However, SOM/clay and SOM/(clay + silt) were not

related with corn S response (Fig. 3), as previously described by Salvagiotti et al. (2005).

The  $N_{an}$  was the best estimation of S response ( $R^2 = 0.62$ ). Values of  $N_{an}$  above  $54 \text{ mg N kg}^{-1}$  resulted in sufficient S availability for corn (Fig. 3a). Similar results were described by Sainz Rozas et al. (2008) when using  $N_{an}$  to predict N availability for corn. These authors reported a  $50 \text{ mg kg}^{-1} N_{an}$  threshold above which corn is unresponsive to N fertilization. Considering the confidence interval for  $N_{an}$  (between 40 and  $69 \text{ mg N kg}^{-1}$ ) (Fig. 3a) we observed that three out of five sites with  $N_{an}$  values below  $40 \text{ mg N kg}^{-1}$  responded to S fertilization, while all sites with  $N_{an}$  values above  $69 \text{ mg N kg}^{-1}$  were unresponsive. This trend indicates that  $N_{an}$  is a good index to predict S response under field conditions, as it was suggested by Wyngaard and Cabrera (2015) from laboratory experiments. Consequently,  $N_{an}$  could be potentially used to simultaneously diagnose S and N availability for corn.

On the other hand, SOM content and  $S_{mineralized}$  showed a weak relationship with corn response to S fertilization (Fig. 3b, c). The S sufficiency thresholds were  $60 \text{ g kg}^{-1}$  and



**Fig. 3** Relationship between S response to fertilization and a mineralizable N determined by short-term anaerobic incubations ( $N_{an}$ ), b mineralizable S determined by short-term aerobic incubations ( $S_{mineralized}$ ), c soil organic matter (SOM), d SOM/clay ratio, and e

SOM/(clay + silt) ratio. Data from 15 experiments. *CI* is the confidence interval of the critical threshold (0.95) and *p* indicates the significance of the regression

1.6 mg S kg<sup>-1</sup> for the SOM content and  $S_{\text{mineralized}}$ , respectively. Above these thresholds, all sites were correctly diagnosed as unresponsive to S fertilization. However, below them nine out of 12 sites were incorrectly diagnosed as responsive to S fertilization for SOM, while the same was observed in 10 out of 13 sites for  $S_{\text{mineralized}}$ . Previous studies did not find a relationship between SOM content and corn response to S fertilization (Salvagiotti et al. 2005; Pagani and Echeverría 2011; Sawyer et al. 2012). Even though available  $\text{SO}_4^{2-}$ -S derives from SOM mineralization, SOM content is not sufficient to explain the S mineralization capacity of different soils, as proposed from laboratory experiments by Tabatabai and Al-Khafaji (1980) and Pirela and Tabatabai (1988).

The weak performance of  $S_{\text{mineralized}}$  as an estimation of S availability can be a consequence of the low range of values resulting from this method (ranging from 0.57 to 1.59 mg kg<sup>-1</sup> in all sites except from site S4) (Table 2). Additionally,  $S_{\text{mineralized}}$  presented a great variability between replicates. The average variation coefficient was 21.3 % for  $S_{\text{mineralized}}$  (data not shown), while it was only 3.8 % for  $N_{\text{an}}$  (data not shown).

The determination coefficient between  $N_{\text{an}}$  and yield response to S fertilization was slightly lower than the one we determined when using  $S_{\text{ini}(0-60)}$  as predictor ( $R^2 = 0.62$  and 0.68, respectively) (Figs. 2b and 3a). This is probably a consequence of  $N_{\text{an}}$  being quantified in the 0 to 20 cm layer, without considering  $\text{SO}_4^{2-}$ -S in the subsoil. However, when comparing at the same depth,  $N_{\text{an}}$  presented a greater determination coefficient than  $S_{\text{ini}(0-20)}$  ( $R^2 = 0.62$  and 0.55, respectively) (Figs. 2a and 3a).

### Incorporation of S mineralization indexes to the $S_{\text{ini}(0-60)}$ model

We fit a quadratic model to predict corn yield response to S fertilization from the traditional diagnostic method  $S_{\text{ini}(0-60)}$  (Table 4). When added to the model, none of the evaluated S mineralization indexes [ $S_{\text{mineralized}}$ ,  $N_{\text{an}}$ , SOM, SOM/clay, SOM/(clay + silt)] improved its predictive capacity (Table 4). This is probably a consequence of the  $S_{\text{ini}(0-60)}$  being already a good predictor of S availability ( $R^2 = 0.63$ , Table 4), limiting

**Table 4** Models to predict S response with different soil variables ( $\text{SO}_4^{2-}$ -S content in soil at sowing 0–60 cm depth ( $S_{\text{ini}(0-60)}$ ), mineralizable S determined by short-term aerobic incubation ( $S_{\text{mineralized}}$ ), mineralizable N determined by short-term anaerobic incubation ( $N_{\text{an}}$ ), soil organic matter (SOM), SOM/clay ratio, and SOM/(clay + silt) ratio

Model	<i>p</i> value	Ra <sup>2</sup>
S response = 2683 + 0.94 $S_{\text{ini}(0-60)}$ § - 98.2 $S_{\text{ini}(0-60)}$ §	0.001	0.63
S response = 2555 + 0.85 $S_{\text{ini}(0-60)}$ § - 89.1 $S_{\text{ini}(0-60)}$ § - 69.2 $S_{\text{mineralized}}$	0.004	0.61
S response = 2557 + 0.86 $S_{\text{ini}(0-60)}$ § - 88.3 $S_{\text{ini}(0-60)}$ § - 2.2 $N_{\text{an}}$	0.003	0.61
S response = 2740 + 0.93 $S_{\text{ini}(0-60)}$ § - 92.4 $S_{\text{ini}(0-60)}$ § - 59.5 SOM	0.002	0.65
S response = 2727 + 0.96 $S_{\text{ini}(0-60)}$ § - 99.3 $S_{\text{ini}(0-60)}$ § - 168.6 SOM/clay	0.004	0.60
S response = 2902 + 1.04 $S_{\text{ini}(0-60)}$ § - 104.8 $S_{\text{ini}(0-60)}$ § - 1471 SOM/(clay + silt)	0.003	0.62

§ parameter is significant at  $p < 0.1$

**Table 5** Coefficient of determination ( $R^2$ ) between  $\text{SO}_4^{2-}$ -S content in soil at sowing 0–60 cm depth ( $S_{\text{ini}(0-60)}$ ) and different soil variables [mineralizable S determined by short-term aerobic incubation ( $S_{\text{mineralized}}$ ), mineralizable N determined by short-term anaerobic incubation ( $N_{\text{an}}$ ), soil organic matter (SOM), SOM/clay ratio, and SOM/(clay + silt) ratio]

	$S_{\text{ini}(0-60)}$	
	$R^2$	<i>p</i> value
$S_{\text{mineralized}}$	0.24	0.062
$N_{\text{an}}$	0.42	0.009
SOM	0.45	0.006
SOM/clay	0.31	0.030
SOM/(clay + silt)	0.16	0.144

the possible contribution of new variables to the model. In addition, most of the evaluated S mineralization indexes were significantly related to  $S_{\text{ini}(0-60)}$ , indicating that these variables account for the same sources of variation (Table 5).

The relationship between  $S_{\text{ini}(0-60)}$  and indexes to estimate S mineralization (Table 5) suggests that in our study most of the  $\text{SO}_4^{2-}$ -S released by mineralization before sowing was accumulated in the soil and was quantified when measuring  $S_{\text{ini}(0-60)}$ . Therefore,  $\text{SO}_4^{2-}$ -S loss mechanisms before sowing, like leaching, were probably negligible. In areas with greater leaching before sowing,  $S_{\text{ini}(0-60)}$  would underestimate soil S availability during the growing season and the incorporation of a S mineralization index to the model would be relevant.

### Conclusions

We identified edaphic properties that effectively predicted S availability for corn, and we determined critical values for these variables. Among them,  $S_{\text{V6}}$  presented a greater predictive capacity than  $S_{\text{ini}}$ , and the performance of both determinations was better when measured at a 0–60 cm depth, rather than a 0–20 cm depth. From the evaluated S mineralization estimations, only  $N_{\text{an}}$  effectively predicted corn yield response to S fertilization. The incorporation of S mineralization indexes to the  $S_{\text{ini}(0-60)}$  model did not improve its performance.



Further research is necessary to validate the critical values we determined and to evaluate the efficiency of incorporating S mineralization indexes to the traditional models under different climate and edaphic conditions, and in different crops.

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