



Sulfur fertilization improves nitrogen use efficiency in wheat by increasing nitrogen uptake

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ABSTRACT

Nitrogen (N) fertilization plays a central role for improving yield in wheat and high N use efficiency (NUE) is desired to protect ground and surface waters. Several studies showed that sulfur (S) fertilization may increase NUE, but no attempts have been made to explain whether this increase is due to greater recovery efficiency (RE), an enhanced internal efficiency (IE) or by an improvement of both efficiencies. The aim of this study was to analyze the effects of different N and S fertilizer rates, and their interaction on N uptake, its partition at maturity, NUE and its main components. Field experiments were carried out during two consecutive growing seasons in the Argentinean Pampas using a single bread-wheat genotype grown under different combinations of N and S fertilizer rates. Additional experiments were performed in farmer fields using N and S fertilization evaluating different genotypes in order to analyze the components of NUE in other environmental conditions. Plant N uptake increased linearly in response to N addition until rates of ca. 80 kg N ha⁻¹. Sulfur addition showed no effect at the lowest N fertilizer rate, but N uptake was increased when S was applied at the highest N rate, revealing a synergism between both nutrients. At the lowest S rate RE was 42%, and increased to 70% when S fertilizer was added. No changes in IE in response to S fertilization were observed. These results were also observed in farmer field experiments, in genotypes that showed different IE. This study showed that S addition increased NUE mainly by increasing the N recovery from the soil. Thus, the concurrent management of N and S is important for reducing the potential pollution of residual soil nitrate by increasing N recovery from the soil while sustaining high nitrogen use efficiency.

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1. Introduction

Nitrogen (N), an essential element of the biochemical processes that drives crop production (Sinclair and Horie, 1989), many times restricts grain yield in wheat grown under field conditions. This is mainly due to a reduction in available N when soil N mineralization is not enough to fulfill the crop demand. Under these circumstances, N fertilization plays a central role for achieving the highest yield in wheat in each particular environment, and a higher N use efficiency (NUE) is desired to protect ground and surface waters (Cassman et al., 2003).

Nitrogen use efficiency indicates how effectively crops transform available N into grain yield, however, to assess the effects of different agronomic practices on this index, it is useful to analyze separately its main components. Typically, these components are

(i) the recovery efficiency (RE), i.e. the ratio between N uptake and applied N, and (ii) the internal efficiency (IE), which reflects the ratio between grain yield (or biomass) and N uptake. Thus, this approach includes different physiological processes that affect N use, as N uptake from the soil, N translocation and N assimilation or redistribution into the plant (Moll et al., 1982; Paponov et al., 1996).

Nitrogen use efficiency may vary when a production factor is changed, e.g. preceding crop (Johnston et al., 1994), tillage system (Sieling et al., 1998) or water availability (Campbell et al., 1993; Timsina et al., 2001). Likewise, numerous reports showed a decline in NUE when N fertilizer rates are increased (Doyle and Holford, 1993; McDonald, 1992; Timsina et al., 2001), since N becomes less limiting at high rates. In an agricultural system in which N supply restricts crop growth, grain yield will increase when N fertilizer is added, but a leveling off may appear if a factor, other than N, e.g. a shortage of other nutrient is limiting. This restriction may be overcome when the deficient nutrient is applied (Aulakh and Malhi, 2004) and, thereby, a change in NUE is expected.

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Sulfur (S) is another essential element in plant nutrition. Particular conditions, such as low organic matter content in soils, soil erosion and high nutrient removal by crops may determine S deficiencies in crops (Scherer, 2001; Tisdale et al., 1986). Salvagiotti and Miralles (2008) showed that S addition increased biomass and grain yield in wheat, reporting a positive interaction between N and S, which was reflected in a greater NUE. Other studies have analyzed the N × S interaction in wheat, but showing results merely at the grain yield level (Randall et al., 1981; Reneau Jr. et al., 1986). As far as we know, no attempts have been made to explain whether the effects of S fertilization on NUE were consequence of changes in RE, IE, or both. Since S is an essential constituent of enzymes involved in N metabolism, i.e. nitrate reductase and nitrite reductase (Campbell, 1999; Mendel, 1997; Swamy et al., 2005), its deficiency could lead to a decrease in N assimilation. Some reports have shown accumulation of nitrates in S deficient plants (Zaroug and Munns, 1980). In S deficient environments, it is expected that a higher N uptake will enhance NUE when S is added.

The aim of this study was to analyze the effects of different N and S fertilizer rates and their interaction on (i) the time course of N uptake during wheat ontogeny and its partition at maturity, and (ii) NUE and its main components.

2. Materials and methods

2.1. General

Two sets of experiments were performed. The first (Experiment set 1) comprised two experimental years for evaluating the effects of S fertilization on the time course of N uptake during the crop cycle and analyze NUE and its components in a Research Farm Station using a single genotype. The second set (Experiment set 2) included experiments performed in farmer fields under different soil conditions than those of Experiment set 1. The objective of this experiment set was to evaluate NUE and its components in other environmental conditions. The description of both sets of experiments is described below.

2.2. Experiment set 1

Two experiments were conducted under field conditions during 2000 and 2001 growing seasons at INTA Research Station, Oliveros, Santa Fe, Argentina (32°33'S, 60°51'W), on a silt loam soil (Typic Argudoll, Serie Maciel). The details of the experiment have been described in a previous paper (Salvagiotti and Miralles, 2008). Briefly, the experiment consisted of four N fertilizer rates in combination with two S rates, arranged as randomized complete blocks in a split-plot design, where N was the main plot and S the subplots, with four and three replications, during 2000 and 2001, respectively. Each plot was 5 m wide and 10 m long. Chemical analysis of the top 20 cm of soil showed an organic carbon content of 13.7 and 13.2 g kg⁻¹, a pH of 5.8 and P Bray content of 20 and 9 mg kg⁻¹ the first and second year, respectively. Nitrate-N content before planting at the same depth was 9 mg kg⁻¹, representing ca. 22 kg N ha⁻¹ both years. Nitrogen and S fertilizers were applied broadcast after planting. Nitrogen fertilizer rates were 26(N₁), 52(N₂), 78(N₃), and 104(N₄) kg N ha⁻¹. These rates were planned to create a large variation of N supply to the crop. The largest N rate was considered to cover the crop N demand for grain yields of ca. 4–5 mg ha⁻¹ (i.e. a requirement of 120–150 kg N ha⁻¹ considering that 30 kg N are needed to produce 1 Mg ha⁻¹ (Ladha et al., 2005)) based on previous studies in the area showing that soil may provide ca. 80–100 kg N ha⁻¹ from mineralization (Salvagiotti et al., 2000). Sulfur treatments consisted of a non-fertilized (S₁) and a fertilized (S₂) treatment, at a rate of 30 kg S ha⁻¹ using

ammonium sulfate. Sulfate-S content (Chesnin and Yien, 1950) (in soil solution plus adsorbed) was 5 mg kg⁻¹, representing ca. 5 kg ha⁻¹. Ammonium sulfate contains 21% N in its formula, thereby N treatments were equilibrated in order to maintain the same N supply within each S treatment. Thus, N₁S₁ treatment included an additional 26 kg N ha⁻¹ as ammonium nitrate and N₁S₂ had 26 kg N ha⁻¹ as ammonium sulfate.

Aboveground biomass was determined from plants harvested in a 0.5-m² area within the central five rows of each plot at the stages of double ridge (DR), terminal spikelet (TS), fully expanded flag leaf (FLE), anthesis (ANT) and physiological maturity (PM) (Tottman, 1986). Wheat plants were clipped at the soil surface and separated into leaves plus stems, spikes, and grains. Then, all samples were dried in an oven at 70 °C for 72 h and weighed. At anthesis, spikes were separated from the rest of biomass (leaves + stems), and at PM, grains were separated from the vegetative structures. Grain yield on a dry-weight basis was determined. Samples were ground and passed through a 0.1-mm mesh, and N concentration in seeds (%N_{seed}) and vegetative structures (%N_{veg}) were determined using a micro Kjeldahl method (Nelson and Sommers, 1973). Nitrogen was quantified colorimetrically using a light spectrophotometer. Nitrogen amount in seeds (SeedN) and in vegetative structures (VegN) was calculated by multiplying N concentration and biomass of each structure separately.

Nitrogen uptake rate (NUR) before anthesis was determined as the slope between N uptake and temperature sum from emergence to each phenological stage. Temperature sum for each developmental stage was calculated as the cumulative mean daily temperature assuming a base temperature of 0 °C (Kirby et al., 1985). At PM, Nitrogen harvest index (NHI, %) was calculated as

$$\text{NHI} = \frac{\text{SeedN}}{(\text{SeedN} + \text{VegN})} \times 100 \quad (1)$$

Linear and linear plateau models were fitted to the relationships between N uptake, grain yield and N fertilizer rate. Nitrogen use efficiency (NUE), i.e. grain production per unit of applied N, was quantified as the slope in the lineal phase of the relationship between grain yield and N fertilizer rate. NUE was decomposed in its components as described by Giller et al. (2004): (i) the N recovery efficiency (RE), i.e. the amount of N taken up by the crop per unit of applied N, estimated through the slope in the lineal phase of the relationship between N uptake and N fertilizer rate, and (ii) the N internal efficiency (IE), i.e. the amount of grain yield per unit of N uptake, quantified as the slope of the relationship between grain yield and N uptake. In order to test the differences between treatments, the parameters of the equations were compared using a *t*-test.

2.3. Experiment set 2

Experiments were conducted in farmer fields in 2001 on three sites in the Rolling Pampas region: Correa (32°51'S, 61°15'W), Oliveros (32°33'S, 60°51'W) and Los Molinos (33°7'S, 61°20'W). Table 1 summarizes soil conditions and management practices in these sites. Grain yield and the economical analysis of these experiments have been previously published by Cordone et al. (2002). The experiments consisted of a factorial combination of two cultivars (Cv): Baguette 10 (B10) and Klein Don Enrique (KDE), and three fertilization treatments (FT): (i) an unfertilized plot (Control C); (ii) 100 kg N ha⁻¹ (N) and (iii) 100 kg N ha⁻¹ plus 20 kg S ha⁻¹ (N + S), arranged in a randomized complete block design with two replications. The N rate used assured that the effects of S fertilization were evaluated under no N deficiency based in N fertilization experiments performed previously in the

Table 1

Characteristics of the sites included in Experiment 2. Soil chemical analysis at planting and planting date for the two wheat cultivars Baguette 10 (B10) and Klein Don Enrique (KDE) are presented.

Site	Soil chemical analysis				Planting date	
	Organic carbon (g kg ⁻¹)	Organic N (g kg ⁻¹)	P (mg kg ⁻¹)	pH	B10	KDE
Oliveros	13.2	1.10	13	5.8	June 22	July 10
Correa	12.8	1.03	12	5.8	July 4	July 4
Los Molinos	11.6	0.96	14	5.8	June 21	July 11

region (Salvagiotti et al., 2004). Nitrogen was applied at planting as urea (46% N) and S as ammonium sulfate, equilibrating both fertilizer sources in order to guarantee the same N rate. In all cases Triple superphosphate was applied in order to avoid any phosphorus deficiency on crop performance. In all cases, the experiments were planted under a no-tillage system on typical Argiudoll soils.

At physiological maturity, in each plot, five plant sub-samples were collected for biomass determination. Each sub-sample consisted of above-ground material from a 0.5-m² area in the center of each plot. Spikes from each sample were separated from the rest of biomass, and grains were harvested by using a static thresher. Each plant part was dried in an oven until constant weight and tissue-N content was measured as described in experiment 1. Grain yield was obtained from a larger harvest area (50 m × 4 m) using a combine. Straw yields (kg ha⁻¹) were estimated from the oven-dry grain yield obtained with the combine and the grain to straw ratio of the 0.5-m² area plant samples taken at physiological maturity. Then total above-ground biomass was calculated as the sum of grain and straw yield (kg ha⁻¹). These estimations were multiplied by grain and straw N concentration determined in the 0.5-m² sub-samples taken at physiological maturity in order to calculate plant N accumulation (kg ha⁻¹).

Since these experiments were performed in farmer fields and using a single N rate, then NUE was determined as

$$\text{NUE} = \frac{\text{GY}_{\text{Fert}} - \text{GY}_{\text{UnFert}}}{\text{N applied}} \quad (2)$$

where GY_{Fert} is grain yield in the fertilized plot (N or N + S) and $\text{GY}_{\text{UnFert}}$ is grain yield in the unfertilized plot. The unfertilized plot was used as the control plot for N and N + S to calculate the efficiency, since previous experiments using S fertilizer without N addition have shown similar grain yields than plots without addition of both N and S (Randall et al., 1990; Pedrol et al., 1999). Following the same reasoning, RE was calculated as

$$\text{RE} = \frac{\text{Nuptake}_{\text{Fert}} - \text{Nuptake}_{\text{UnFert}}}{\text{N applied}} \quad (3)$$

where $\text{Nuptake}_{\text{Fert}}$ is N uptake in the fertilized plot (N or N + S) and $\text{Nuptake}_{\text{UnFert}}$ is N uptake in the unfertilized plot (N or N + S). The internal efficiency was calculated as

$$\text{IE} = \frac{(\text{GY}_{\text{Fert}} - \text{GY}_{\text{UnFert}})}{(\text{Nuptake}_{\text{Fert}} - \text{Nuptake}_{\text{UnFert}})} \quad (4)$$

2.4. Data analysis

Experiments were analyzed using the PROC MIXED procedure (Littell et al., 1996). Nitrogen, S, year, and their interactions were analyzed in Experiment set 1. To test the year and N effects, replication (year), and replication × N (year) were used as error terms, respectively (Gómez and Gómez, 1986). In Experiment set 2,

the effects of site, cultivar and fertilization and the interactions were analyzed using replication (site) was used as an error term to evaluate the site effect (Gómez and Gómez, 1986). When interaction was not statistically significant ($P > 0.05$), data were pooled and main effects were discussed.

3. Results

3.1. Experiment set 1

3.1.1. Time course of N uptake

Depending on the period considered in the crop cycle, the addition of S increased N uptake from 5 to 7% and from 13 to 16% in 2000 and 2001, respectively. However, the relative increases in N uptake in response to N fertilization fluctuated from 27 to 70% and from 25 to 53% for the first and second year, respectively (Table 2). A significant interaction between both nutrients, N and S, was observed early in the crop cycle, as S addition showed no effect at the lowest N fertilizer rate, but when N became less limiting, a greater N uptake was observed at the highest S rate (Table 2).

Although the average N uptake at PM was similar in both seasons, ca. 100 kg N ha⁻¹ (Table 2), the time course of N uptake during the crop cycle between years was different. Averaging both S rates, N uptake at anthesis ranged between 57 and 80 kg N ha⁻¹ in 2000 and between 76 and 100 kg N ha⁻¹ in 2001. In the same way, N uptake between anthesis and PM ranged from 18 to 40 kg N ha⁻¹ in 2000 being lower in 2001, i.e. 8–18 kg N ha⁻¹. Therefore, in 2000 ca. 70% of N uptake at PM was absorbed at anthesis, while in 2001 this proportion was ca. 90% (Table 2). Consequently, the magnitude of N translocation from vegetative structures to the grain (N uptake at anthesis minus N in vegetative structures at PM), was different both years. While in 2000, N translocation from vegetative structures to the grain varied from 32 to 40 kg ha⁻¹ (i.e. grains contained ca. 40–60% of N uptake at PM), in 2001, this proportion was larger, ca. 70–80% of N taken up after anthesis was contained in grains, representing 50–60 kg ha⁻¹.

The maximum NUR was observed before anthesis (i.e. between DR and FLE), and the highest values were obtained at N rates above 80 kg N ha⁻¹ (i.e. N₃ Treatment). In the same way, increments in NUR due to the addition of S were observed at N fertilizer rates above 80 kg ha⁻¹. The effect was significant only in the first growing season; however the same trend was observed in the second year of experiments. On average, above 80 kg N ha⁻¹, without S addition NUR was 6.7 g N m⁻² per degree day, but increased 22% when S was added. No S effects on NUR were observed at the lowest N rate (Fig. 1).

Throughout the crop ontogeny, and with the exception of the TS stage, larger N recovery efficiency, i.e. kg N taken up by the crop per unit of fertilizer N applied, was observed at the highest S supply. From DR to anthesis, S addition increased this recovery efficiency from 11 to 83% in 2000 and from 24 to 76% in 2001 (Table 3).

3.1.2. N uptake at physiological maturity and Nitrogen use efficiency

At physiological maturity, N uptake showed a significant N × S interaction, however, the N × S × year interaction was not statistically significant (Table 2). Sulfur addition did not increase N uptake at the lowest N fertilizer rate, but the response to S was evident as N fertilizer rate increased. Thus, at S₁, N uptake at PM increased from 9% at N₂ to 27% at N₄, but at S₂ this relative increase ranged from 35% at N₂ to 55% at N₄ (Table 2). For both S rates, N uptake increased linearly in response to N addition until N fertilizer rate was ca. 80 kg ha⁻¹, and the trend suggests no further increases in N uptake above this rate (Fig. 2C), thus a lineal plateau model was fitted. The slope of this relationship, i.e. RE, was significantly different as a consequence of the different S rates application. At the lowest S rate, the crop recovered 42% of

Table 2

Nitrogen uptake (kg ha^{-1}) and ANOVA *F*-test probabilities for the effects of N, S, year and their interaction effects at different ontogenic stages (DR = double ridge, TS = terminal spikelet, FLE = flag leaf expanded, Ant = anthesis, PM = physiological maturity^a) (SE = standard error of the mean).

N levels	DR		TS		FLE		Ant		PM	
	S ₁	S ₂								
2000										
N ₁	21	22	23	28	48	46	59	55	81	73
N ₂	25	24	34	35	47	49	66	72	89	105
N ₃	24	24	38	41	67	73	73	77	101	117
N ₄	26	33	42	40	65	75	76	84	111	123
SE N	1.99		3.04		4.28		5.10		7.96	
SE S	1.20		1.83		2.51		2.93		4.10	
2001										
N ₁	13	14	55	67	71	76	73	79	85	89
N ₂	12	16	64	74	75	90	82	105	96	113
N ₃	18	20	73	77	92	107	95	108	109	118
N ₄	17	17	64	91	83	105	83	116	101	128
SE N	1.29		4.16		3.36		3.61		4.06	
SE S	0.72		2.45		2.25		2.20		2.95	
<i>P</i> <										
Year	0.006		<0.001		<0.001		0.002		0.320	
N	0.005		0.002		<0.001		<0.001		0.002	
S	0.031		0.0002		<0.001		<0.001		<0.001	
N × S	0.626		0.299		0.108		0.021		0.007	
Year × N	0.070		0.997		0.663		0.783		0.843	
Year × S	0.941		0.002		0.015		0.001		0.204	
Year × N × S	0.114		0.040		0.958		0.484		0.314	

^a The values of total N uptake at PM (SeedN + VegN) were splitted by year because the interaction year*N or year*S was significant in other phenological stages.

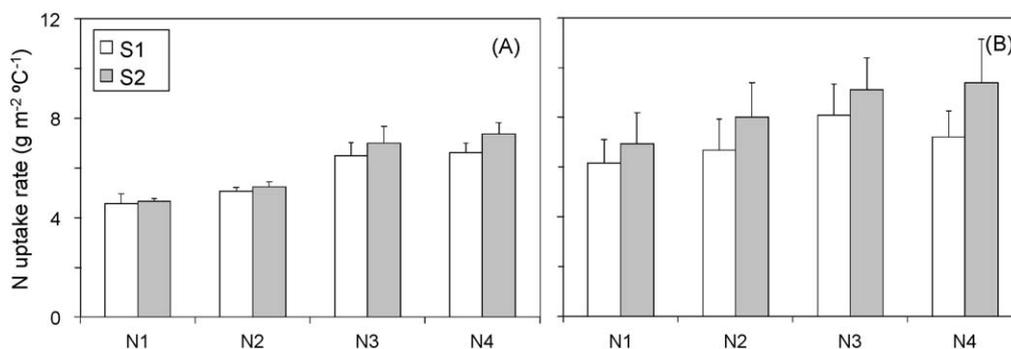


Fig. 1. Nitrogen uptake rate before anthesis (between DR and FLE) under two sulfur (S) and four nitrogen (N) fertilization rates during two growing seasons (2000, panel A, and 2001, panel B). Vertical segments represent the standard error of means.

fertilizer N per each unit of N added to the soil, but when S was added, RE was increased by 66% and ca. 70% of the applied N was recovered by the crop (Fig. 2C).

Nitrogen uptake at PM was linearly related to grain yield. The slope of this relationship (i.e. IE) was not significantly affected by different S rates (Fig. 2B), so a common internal efficiency was found (i.e. 22.5 kg grain per kg of N uptake) for both sulfur rates.

Nitrogen use efficiency, i.e., kg grain per unit N fertilizer rate, was ca. 50% higher when the crop received S fertilizer than when the crop did not receive S fertilizer (Fig. 2A). Consistently with that observed in RE, and at least for this data set, grain yield did not show increments for both S rates when N fertilizer rates were higher than 80 kg N ha^{-1} . Therefore, NUE for N fertilizer rates between 20 and 80 kg N ha^{-1} were ca. 11 and 16 kg grain per kg of fertilizer N for S₁ and S₂ sulfur rates, respectively (Fig. 2A).

Nitrogen and S addition significantly increased the amount of N both in seeds and in vegetative structures explained by increases not only in biomass but also in the N concentration (Table 4). In S₁, N addition increased SeedN by 15% on average, whereas this

increment was doubled at S₂, i.e. 31%. Conversely, the relative increase in VegN due to N addition was higher, i.e. 33 and 72%, for S₁ and S₂, respectively. On average, at N rates above N₂, S fertilization increased SeedN and VegN by 10 and 29%, respectively (Table 4).

Nitrogen fertilization also increased N concentration in seeds, but a slight increase was observed when S was added. The larger effects of N addition on N concentration in seeds were observed when N fertilizer rate was above 50 kg N ha^{-1} without significant increases between N rates. When S was added, these differences were greater, increasing N concentration in seed by 8% on average (Table 4). In vegetative tissues, the relative effect of S addition was larger. Thus, with the exception of the N₁ treatment where S addition did not show significant changes in N concentration, the increase in S supply boosted N concentration in vegetative tissues by 18% (Table 4).

Changes in N content in the different plant compartments were reflected in NHI. On average, 65% of N was allocated in the seeds. Nitrogen harvest index was significantly reduced at N rates above

Table 3

Recovery efficiency (\pm standard error) (kg N uptake kg fertilizer N ha⁻¹) at different ontogenic stages during the crop cycle: double ridge (DR), terminal spikelet (TS), flag leaf expanded (FLE) and anthesis (Ant), for two sulfur (S) fertilization levels (S₁ and S₂) during the 2000 and 2001 growing seasons. The efficiency was calculated in the lineal phase of N uptake increase (between N₁ and N₃).

S treatment	DR	TS	FLE	Ant
2000				
S ₁	0.06 \pm 0.04	0.29 \pm 0.06	0.37 \pm 0.14	0.27 \pm 0.03
S ₂	0.04 \pm 0.02	0.25 \pm 0.04	0.52 \pm 0.14	0.42 \pm 0.10
2001				
S ₁	0.10 \pm 0.06	0.34 \pm 0.06	0.40 \pm 0.04	0.42 \pm 0.04
S ₂	0.12 \pm 0.003	0.19 \pm 0.02	0.59 \pm 0.03	0.56 \pm 0.16

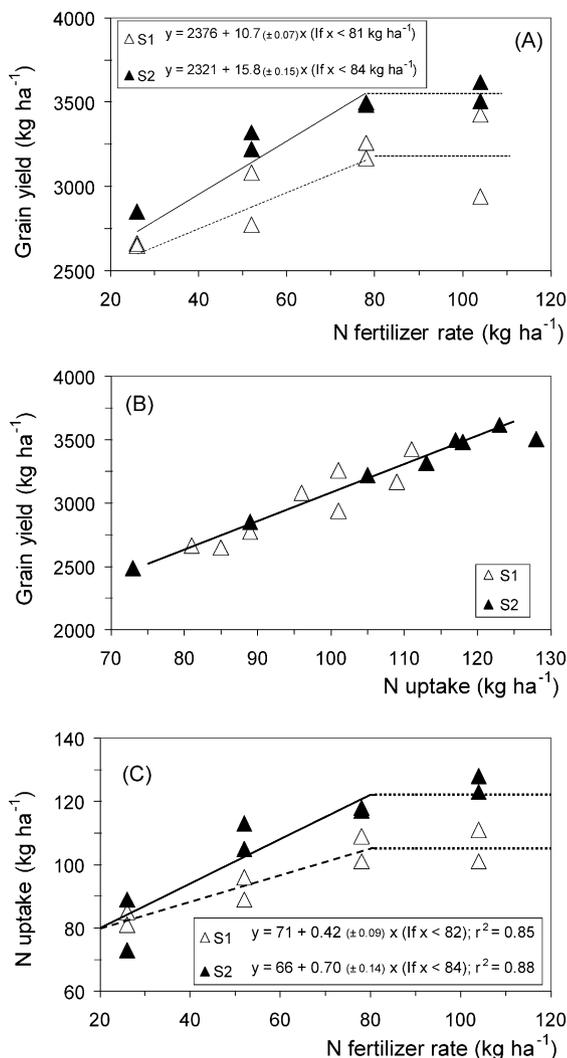


Fig. 2. Relationship between grain yield and (A) N fertilizer rate and (B) N uptake at physiological maturity and (C) N uptake at physiological maturity and N fertilizer rate of wheat grown under two sulfur fertilization rates (S₁ and S₂). Data from panel A are adapted from Salvagiotti and Miralles (2008). Solid and tagged lines in figures A and C represent the linear regression for S₂ and S₁, respectively. In panel B, solid line represents the linear regression for both S₁ and S₂.

80 kg N ha⁻¹ (N₃) by 7% and 10% in S₁ and S₂ respectively. On average, S addition reduced NHI ca. 7% above N₂ (Table 4).

3.2. Experiment set 2

Sulfur fertilization increased grain yield and N uptake by 7 and 22%, respectively in the experiments conducted in farmer fields, showing a significant cultivar \times site interaction (Table 5).

Nitrogen use efficiency varied between experimental sites, from 4.6 to 14.3 kg grain per kg of applied N, and a significant 30% increase in NUE was observed when S was added to the crop (Table 5). Recovery efficiency range from 27 to 56% of applied N in the different environments, and S addition showed a 30% average increase. On the other hand, no differences in response to S addition were observed in IE. However, this component of NUE showed significant differences when comparing genotypes. Klein Don Enrique showed a 13% increase in IE over Baguette 10 (Table 5). No changes were observed in harvest index between cultivar, site and fertilizer treatment (0.30 on average, data not shown).

4. Discussion

As expected, a greater N uptake was observed when N fertilizer rate increased. However, the results showed an evident N \times S synergism since the addition of S boosted N uptake as N fertilizer rates increased. In contrast, S addition showed no significant effects at the lowest N increment, suggesting that soil S may have been enough to meet the crop N demand at this N uptake level. This interaction was also observed when analyzing biomass and grain yield in the same experiment (Salvagiotti and Miralles, 2008), and in previously reported studies of S fertilization in grasses (Brown et al., 2000). The time course of N uptake (expressed in terms of the proportion of total N uptake reached at anthesis) was different depending on the growing season, even when the amount of N taken up by the crop at the end of the cycle was similar in both years. Environmental conditions during the stem elongation period (i.e. from terminal spikelet to anthesis) may affect crop growth and thus, modify the pattern of N uptake (Baethgen and Alley, 1989; Fischer, 1993; Hocking, 1994). The larger mean air temperature during stem elongation observed in the second year of experimentation (15 °C in 2000 vs. 17 °C in 2001) could accelerate the rate of crop development affecting crop N uptake rate (see Fig. 1), and thereby modifying the time course of N uptake.

At physiological maturity, NHI is an indicator of the magnitude of N remobilization from vegetative tissues. In the present study a larger NHI was observed when N became more limiting for crop growth, suggesting a greater N remobilization. This remobilization may have reduced the N content in leaves determining a concomitant reduction in the activity of the ribulose biphosphate carboxylase/oxygenase enzyme (Rubisco) during the leaf senescence period (Irving and Robinson, 2006; Millard, 1988). Thus, the drop in Rubisco activity may have reduced CO₂ assimilation and thereby decreased the biomass production.

Sulfur addition consistently decreased NHI, indicating that S deficient plants mobilized more N to the grains. Therefore, the increase in biomass observed in response to S addition in Salvagiotti and Miralles (2008) can be explained in terms of a greater accumulation of N in vegetative tissues, i.e. stored as Rubisco in leaves. As no change in grain N concentration was observed, the accumulation of N in grains may have been proportional to biomass production. Then, the larger N accumulation in the grains due to S addition was explained by increases in grain biomass, rather than changes in grain N concentration.

In the present study, the N rate above which no increases in grain yield were observed was ca. 80 kg N ha⁻¹ (Salvagiotti and Miralles, 2008). This threshold is within the range of values reported in previous N fertilization studies in the area that showed no grain yield increases above 90 kg N ha⁻¹ (summing N-NO₃ in soil + fertilizer) (Salvagiotti et al., 2004). Some studies have shown a rise in grain N concentration when a crop is grown above this non-limiting N supply (Uhart and Andrade, 1995; Lazzari et al., 2001; Gooding et al., 2007a,b). Contrasting to these published evidence, the present study did not show changes in N

Table 4

Mean values and ANOVA *F*-test probabilities of N accumulated in seeds (SeedN) and in vegetative tissues (vegN), N harvest index (NHI), N concentration in the seed (%NSeed) and in vegetative tissues (%NVeg), at physiological maturity in wheat crops grown under different supply of N and S. Each value is the mean of two seasons^a (SE = standard error of the mean).

N levels	SeedN		VegN		NHI		%NSeed		%NVeg	
	kg ha ⁻¹				%		g kg ⁻¹			
	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂
N ₁	57	55	26	26	69	67	19.2	18.7	5.7	5.7
N ₂	61	69	29	40	67	63	20.2	22.4	6.2	7.6
N ₃	68	71	37	46	65	60	22.0	23.7	6.4	7.6
N ₄	68	76	38	48	64	61	21.6	22.4	6.8	7.8
SE N	3.19		2.57		1.43		1.3		0.5	
SE S	1.78		1.46		1.15		1.0		0.4	
	<i>P</i> <									
Year	0.900		0.208		0.334		0.903		0.321	
N	0.010		0.001		0.013		0.102		0.030	
S	0.011		<0.001		<0.001		0.154		<0.001	
N × S	0.058		0.008		0.280		0.540		0.209	
Year × N	0.637		0.960		0.532		0.457		0.478	
Year × S	0.516		0.115		0.243		0.379		0.308	
Year × N × S	0.442		0.121		0.096		0.877		0.122	

^a SeedN and VegN at physiological maturity (PM) correspond to the average of two years because the year*N and year*S interactions were not significant.

Table 5

Mean values and ANOVA *F*-test probabilities of grain yield, total nitrogen (N) uptake, N use efficiency (NUE), N recovery efficiency (RE) and N internal efficiency (IE) of two wheat cultivars (Cv) Baguette 10 (B10) and Klein Don Enrique (KDE) in three sites with different N and sulfur (S) fertilization treatments (FT) (C: Control unfertilized treatment) (SE = standard error of the mean) (Experimental set 2).

Site	Cv	Grain yield			Nitrogen uptake			NUE		RE		IE	
		kg ha ⁻¹			kg ha ⁻¹			kg grain kg applied N ⁻¹		kg N uptake kg applied N ⁻¹		kg grain kg uptake ⁻¹	
		C	N	N + S	C	N	N + S	N	N + S	N	N + S	N	N + S
Los Molinos	B10	2900	4309	4394	54	101	111	12.2	13.0	0.47	0.57	26	23
	KDE	2446	4124	4440	48	99	118	14.6	17.3	0.51	0.70	29	25
Oliveros	B10	3265	4265	4481	93	137	145	8.7	10.6	0.31	0.38	28	28
	KDE	2225	3185	3520	69	108	117	8.3	11.3	0.39	0.47	22	24
Correa	B10	3072	3300	3776	95	111	133	2.0	6.1	0.17	0.38	12	16
	KDE	2794	3301	3452	86	109	115	4.4	5.7	0.23	0.29	19	19
SE Site		112			4.8			1.8		0.04		4.0	
SE Cv		89			3.2			1.0		0.02		2.4	
SE FT		77			3.0			1.01		0.02		2.4	
	<i>P</i> <												
Site		0.08			0.07			0.04		0.01		0.40	
Cv		<0.001			<0.001			0.02		0.54		0.02	
FT		<0.001			<0.001			0.002		<0.001		0.70	
Cv × FT		0.68			0.92			0.96		0.72		0.92	
Site × FT		0.005			0.003			0.79		0.54		0.08	
Site × Cv		0.01			0.004			0.17		0.20		0.18	
Site × Cv × FT		0.78			0.34			0.24		0.17		0.41	

concentration in grains when N fertilization go beyond the maximum grain yield. However, and in line with other studies in wheat (Cassman et al., 1992; Ehdai and Waines, 2001), an increase in N concentration in vegetative tissues was observed when N fertilizers rates increased over 80 kg N ha⁻¹, suggesting that part on nitrogen absorbed by the crop was not partitioned to the sink during the grain filling period.

Nitrogen use efficiency increased when S fertilizer was added. However, this variable is not useful to understand the impact of crop management strategies (e.g. S fertilization) because it encompasses both, soil and plant processes. The analysis of RE, more related to processes that occur in the soil, and IE, closely associated with plant processes, improves the interpretation of the results because comprises a description of those processes likely associated with changes in NUE.

Both set of experiments clearly showed a greater N uptake when S fertilizer was added, without altering the internal efficiency. Then, these results showed that variations in NUE in response to S fertilization were more associated with changes in RE than in IE, in agreement with previous studies in wheat (McDonald, 1992) and in rice (Cassman et al., 2003). Hence, processes in the soil associated with N availability had a dominating influence on NUE due to variations in S supply. Giller et al. (2004) suggest that RE may vary as a consequence of changes in the assimilation of N at the root surface or by root induced changes in the rhizosphere. However, in the present experiment, it is more likely that the addition of S, which increased N uptake, acted by promoting a larger volume of soil explored by the roots, as was previously reported in studies manipulating N fertilization in barley (Atkinson, 1990) and wheat (Katterer et al., 1993; Mandal et al.,

2003). Considering that roots represent a proportion of aerial biomass, i.e. between 4 and 48% depending on the phenological stage (Katterer et al., 1993), the greater aerial biomass observed in response to N and S fertilization reported by Salvagiotti and Miralles (2008) could have led to a concomitant increase in root biomass allowing to capture more N from the soil, and thus increase N uptake per unit of N fertilizer added (i.e. RE). The data of the present study (see Experiment set 1) showed a rise in N uptake rate and RE in response to S fertilization from TS to anthesis, in coincidence with a period in which the requirement of N and other nutrients from sinks are increasing (Abbate et al., 1995; Demotes-Mainard et al., 1999). This information indicates that N and S should not be scarce in this period in order to improve RE.

The fact that S fertilization did not modify the internal N use efficiency is not surprising, since variables that may explain the variations in IE, such as grain N concentration or harvest index (Giller et al., 2004; Ladha et al., 2005), were not modified by S addition (Salvagiotti and Miralles, 2008). In fact, IE was nearly constant over a range of environmental conditions (see Experiment set 2). Similar values of IE found in the present study, have been observed in wheat crops grown under different environmental conditions (Doyle and Holford, 1993; Duivenbooden et al., 1996; Novoa and Loomis, 1981; Timsina et al., 2001), suggesting that this component is more conservative, i.e. present low variation under different environmental conditions, than RE. This observation is in line with other reports showing no significant changes in IE when a factor of production was modified (Muurinen et al., 2006; Paponov et al., 1996).

In summary, the increase in grain yield in response to S addition was associated with a higher N uptake rate before anthesis confirming the positive interaction between both nutrients. These results, which as far as we know are the first that describe the effects of S on different components of NUE, showed that S fertilization affected soil processes associated with NUE. In contrast, plant processes related to this variable were fairly stable. From an environmental point of view, a higher RE is a desired feature associated with a higher capacity of the crop to capture N from the soil (Alva et al., 2005). Therefore, this study shows that the concurrent management of N and S is important for reducing the potential pollution of residual soil nitrate by increasing N recovery from the soil and sustaining high nitrogen use efficiency.

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