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SOIL SULFUR AND NITROGEN AVAILABILITY IMPROVES ROOT BIOMASS GROWTH OF RYEGRASS (*Lolium multiflorum* L.)

DISPONIBILIDAD DE AZUFRE Y NITRÓGENO EN EL SUELO MEJORA LA PRODUCCIÓN DE BIOMASA RADICAL DE BALLICA (*Lolium multiflorum* L.)

Carolin Córdova[1]*, Ana María Martínez[1], Ángela Machuca[3], Erick Zagal[1], Susana Fischer[2], Matías Betancur[1]

[1] Department of Soils and Natural Resources, Faculty of Agronomy, University of Concepción, Avenida Vicente Méndez 595, Chillán, Chile.
[2] Department of Plant Science, Faculty of Agronomy, University of Concepción, Avenida Vicente Méndez 595, Chillán, Chile.
[3] Department of Science and Technology, School of Science and Technology, University of Concepción, Juan Antonio Coloma 0201, Los Ángeles. Chile.
*Corresponding author E-email: carolin.cordova@gmail.com

RESUMEN

Recientemente se ha detectado deficiencia de azufre (S) en los suelos, lo cual afecta la productividad de los cultivos y la absorción de nitrógeno (N). El objetivo de este estudio fue evaluar el efecto de la aplicación de S al suelo sobre la eficiencia en la recuperación de N. En dos suelos, Andisol y Mollisol, se determinó la mineralización de N y S, el N potencialmente disponible, y la actividad de ureasa. Para los parámetros microbiológicos no se encontraron diferencias ($P \leq 0,05$) entre los suelos, pero la cinética de mineralización del suelo Andisol mostró un pequeño aumento en el tiempo. En el suelo Mollisol se realizó un experimento en macetas sembradas con ballica (*Lolium multiflorum* L.) con 4 tratamientos: control, sólo S, sólo N, y N más S, en dosis equivalente a 30 kg S y 100 kg N ha^{-1}, por un período de crecimiento de 16 semanas, para luego determinar materia seca total, concentración de N, y eficiencia de recuperación de N. Se registró un incremento del 26% en la recuperación de N como resultado de la aplicación de S, pero no fue significativamente diferente del tratamiento control ($P \geq 0,05$). La absorción de S en la biomasa del cultivo fue baja, evidenciado en la alta concentración residual de S del suelo. Sin embargo, la aplicación conjunta de N y S mostró un incremento ($P \leq 0,05$) en la producción radical del cultivo.

Palabras clave: eficiencia nitrógeno, mineralización azufre, mineralización nitrógeno, sinergismo NxS, Mollisol, Andisol

ABSTRACT

Soil sulfur (S) deficiencies have been detected lately, affecting crop production and nitrogen (N) uptake. The objective of this study was to assess the effect of S application on crop N recovery efficiency. N and S mineralization, potentially available N, and urease activity were measured in Andisol and Mollisol soils. No differences were found ($P \leq 0.05$) between the soils in terms of microbiological parameters, but soil mineralization kinetics showed a small increase in the Andisol soil. The Mollisol soil was used to carry out a pot assay with rye grass (*Lolium multiflorum*), including

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four treatments: S, N, the combined application of S and N at a rates of 30 kg S ha^{-1} and 100 kg N ha^{-1}, and a control treatment. Crop biomass dry matter, N concentration, and N recovery efficiency were determined sixteen weeks after sowing. N recovery efficiency increased by 26% with S application, but it was not significantly different from the control treatment ($P \geq 0.05$). The S content in the crop biomass was low, and a large residual soil S was observed at the end of crop growth. However, the combined application of N and S resulted in an increase ($P \leq 0.05$) in the root growth of plants.

Key words: nitrogen recovery efficiency, sulfur mineralization, nitrogen mineralization, synergism NxS, Mollisol, Andisol

INTRODUCTION

Soil sulfur (S) is an essential nutrient for crop growth because it is part of essential amino acids, which are important not only for plant development (Thomas et al., 2003) but also for animal nutrition (Kahindi et al., 2017). Crop production benefits from an adequate S supply, which can also improve plant N metabolism (Hawkesford et al., 2012) because S catalyzes chlorophyll production, improving the efficiency of the photosynthesis process. An adequate plant S nutritional status may improve crop yield (Raza et al., 2018), protein and amino acid contents (Hawkesford et al., 2012), hordeins composition (Prystupa et al., 2019), and gluten content (Tao et al., 2018), which are all important parameters to global food security (Prosekov and Ivanova, 2018).

Sulphur deficiencies in soil have been reported in the last decade (Scherer, 2001), but the issue has not been widely investigated in Chile. Early reports described S as the second most available element in local volcanic soils (Schenkel et al. 1973), while a later study conducted by Alfaro et al. (2006) reported soil S deficiencies. Based on this, it is possible to indicate that S has become deficient in volcanic soils of the South of Chile in a period of 30 years.

Apart from its essential role in plant growth, S can also influence N uptake because both elements are required for protein synthesis (Salvagiotti et al., 2009; Arshad et al., 2010). For example, the relationship between N and S in protein content is N/S = 40 for cereals, and N/S = 30 for legumes. Therefore, crop production benefits from the effect of S, which is usually also linked to N (Hawkesford et al., 2012). Thus, an adequate supply of available S in the soil might benefit crop N use efficiency since S and N cycles in the soil have some common characteristics (Eriksen, 2009). However, most farmers are not aware of the importance of S application to the soil to improve crop yield and N use efficiency.

Soils with high organic matter content, such as Andisol and Mollisol soils, are desirable for crop production due to their high chemical and physical fertility. However, this does not guarantee an adequate supply of plant nutrients from organic matter through the mineralization process, while fertilizer application is usually needed to meet crop requirements. Data on S mineralization in Andisol soils is particularly scarce, leading to a gap in the understanding of natural soil supply of S.

The current global scenario requires efficiency of N application to improve agricultural management and decrease environmental impact. S has a key role on N plant metabolism as it can improve N use efficiency. Therefore, the objective of this research was to evaluate S supply from soils with high organic matter content and assess the effect of S availability on crop N uptake. This was determined by calculating N recovery efficiency using a short-term crop growth under soil S deficiency.

MATERIALS AND METHODS

Experimental site, soil sampling and conditioning. The study site (36°35’44’’ S; 72°04’36.25’’ W) has been previously described by Sanchez-Hernández et al. (2017). Samples were taken at 0-20 cm depth from an Andisol and a Mollisol soil (Table 1). The Andisol soil was under a wheat-maize crop rotation, while the Mollisol soil was under a four-year-old alfalfa pasture.

A completely randomized design was used (n = 3). To assure randomization, the sampling site was divided in plots of 30 m by 30 m using Google Earth satellite images, and three plots were randomly sampled. Soil samples were composited within the plots, transported to the lab, and sieved at 10 mm for a pot assay and then at 2 mm for microbiological soil analyses.

Microbiological soil indicators.

Soil carbon and nitrogen mineralization. Carbon (C) and N mineralization were measured by soil incubation in an enclosed system (Anderson, 1982), while an open incubation system was used (Maynard et al., 1983) to determine S mineralization. Soil samples were incubated at 22ºC for 112 days, but partial measurements were also made at 7, 14, 28, and 56 days, respectively. Field replicates were worked in triplicate in the

Table 1. Chemical properties of two cultivated soils with limited nitrogen (N) and sulfur (S) availability.
Tabla 1. Propiedades químicas de dos suelos cultivados con disponibilidad restringida de nitrógeno (N) y azufre (S).

Soil properties	Mollisol		Andisol	
	Value	Level	Value	Level
Soil pH	5.9	Low	5.9	Low
Soil organic matter (%)	3.5	Medium	5.7	Medium
Available phosphorous (mg kg^{-1})	28.3	High	18.6	Medium
Available potasium (mg kg^{-1})	79.23	Low	411.3	High
Available N-NO_3^- (mg kg^{-1})	7.5	Low	14.0	Low
Available N-NH_4^+ (mg kg^{-1})	2.8	Low	3.2	Low
Available sulphur (mg kg^{-1})	4.0	Low	8.7	Low

lab, and a control treatment was used for all measured parameters.

Soil C mineralization was measured as CO_2–C produced by microbial respiration, using 25 g of soil incubated with a trap of 10 mL of NaOH (0.01 M), which was replaced at each evaluation period. The excess of NaOH after reacting with $BaCl_2$ (0.5 M) was titrated with an HCl solution (0.1 N) (Anderson, 1982)

Soil N mineralization was assessed by measuring the NH_4^+-N content from 7 g of soil extracted with KCl (1 M) at a ratio of 1:5 soil: solution, shaken for 1 h, at each stage of the temporal evaluation. Colorimetric analysis was conducted after the addition of 3 mL NaOH (3 M) and 2 mL of Nessler reactive, by spectrophotometry (490 nm) (Maynard et al., 1983).

S mineralization was quantified in a 150 mL $CaCl_2$ solution (10 mM) applied to an open system, consisting of a Büchner funnel containing 30 g of soil. Sulphate concentration was measured through the ion chromatography technique using 1 mL of the extracting solution (Córdova et al., 2017).

Potentially available N. This indicator of the seasonal provision of soil available N for crop growth was determined by measuring the difference between the initial soil ammonium content, and that measured at the end of a 7-day soil incubation period at 40°C under anaerobic conditions. The soil ammonium content was extracted on 5 g of dry soil and 25 mL KCl (2 M), followed by colorimetric analysis and spectrophotometric determination.

Urease activity. Four mL of phosphate buffer solution and urea (6.4%) were added to fresh samples of 1 g of soil. The mixture was shaken and then the system was incubated for 2 h at 37°C using a temperature controlled water bath. After cooling down, 5 mL of KCl (2 M) solution were added and, from the filtered extract, 2 mL were combined with EDTA reactive, 2 mL of phenol-nitroprusside, 4 mL of hypochlorite buffer and deionized water to reach 25 mL. The solution was incubated at 40°C for 30 min, and then the absorbance was measured by spectrophotometry at 636 nm. NH_3-N concentration was obtained from calibration using standard solutions of 0, 25, 50, 100 and 150 μg NH_3-N (Sastre and Lobo, 2003)

Crop growth assay

A Mollisol soil was selected to establish a pot experiment and measure the effect of S application on plant N recovery. Two levels of N application combined with a rate of S equivalent to 30 kg ha^{-1} were allocated in pots containing 1 kg of soil (Table 2), and the nutrients were applied as a solution of NH_4NO_3, K_2SO_4, and KCl. Each pot was sown with 1 g of *Lolium multiflorum* L. Soil moisture was kept at 50% of the total water availability, monitored using a calibrated moisture sensor, while weeds were manually removed every week. The total number of experimental units were n = 36 (4 treatments, 3 field replicates, 3 greenhouse replicates), arranged in a randomized design. They were kept under greenhouse conditions for 16 weeks.

Soil available N. Soil nitrate was measured at the beginning and at the end of the crop growth period. Nitrate content was determined by soil extraction using deionized water in a ratio of 1:4 soil:solution, shaken, and the concentration was obtained by ion chromatography.

Soil available S. Soil available sulphate was measured twice during the experiment. A

Table 2. Equivalent rates of the combined application of sulfur (S) and nitrogen (S) to the soil used to determine the effect of S application on plant N recovery in a pot experiment.

Tabla 2. Tasas equivalentes de aplicación combinada de azufre (S) y nitrógeno (N) al suelo usadas para determinar el efecto de la aplicación de S sobre la recuperación de N en un ensayo en macetas.

Treatments	S application (kg ha^{-1})	N application (kg ha^{-1})
N0S0	0	0
N0S30	30	0
N100S0	0	100
N100S30	30	100

solution of Ca $(H_2PO_4)_2$ (0.01 M) was applied to a dry soil sample of 5 g, and the mixture was shaken for 30 min. After filtration, an aliquot of 1 mL was used to measure sulphate concentration by ion chromatography.

Crop growth. Crop height (cm) was recorded on a weekly basis.

Crop yield. The biomass produced by the crop was harvested at the end of the growth period, and foliage was separated from the roots, cleaned and kept in disposable paper bags. The bags were introduced in an oven at 65°C and weight was registered (mg) after 24 h (constant weight).

Foliage total N. After weighting the foliage dry matter, the samples were milled and sieved at 1 mm, and an amount of 0.5 g was used to determine N concentration (Sadzawka et al., 2007). A composite solution using sulphuric acid, salicylic acid, and hydrogen peroxide, was added to the foliage dry matter and left to stand overnight. Each sample was heated up until digestion, and then the NH_4^+-N was determined by distillation and titration.

Nitrogen recovery efficiency. This was calculated as the ratio between the difference of fertilized pots (N100S0, N100S30) and control treatments (N0S0, N0S30), and the plant available N estimated from initial nitrate content and the applied N.

Data analysis.

Soil and crop variables from the experimental randomized design applied were analyzed using the F test ($P \leq 0.05$), and after tested for normality, and homogeneity of variances. The statistical differences between the treatments were assessed applying the Tukey test. The data were analyzed using SAS Windows v8 package (SAS Institute Inc., 1999)

RESULTS AND DISCUSION

Soil S and N supply

Andisol and Mollisol soils were contrasted in their chemical properties and capacity to provide N and S to the soil solution. Soil pH was identical for both soils, but the Andisol showed better fertility (the level of organic matter was higher), as well as greater N and S availability (Table 1). However, these initial values of soil S and N are low for Andisol and Mollisol soils, and therefore they had potential to respond to the application of S and N. The analysis of the mineralization process (C, N, and S) indicates the supply of these nutrients from the soil.

In general, the C mineralized from Andisol and Mollisol soils did not show differences ($P \geq 0.05$) up to 56 days of soil incubation, but the Andisol registered a higher value ($P \leq 0.05$) at the end of the incubation period (112 days) (Fig. 1A and B). C mineralization in the Mollisol soil reached a maximum rate between 14 and 28 days of incubation ($P \leq 0.05$), whilst the Andisol soil did not show significant differences ($P \geq 0.05$) between temporal rates of C mineralization (Table 3). This might be explained by the higher organic matter content observed in the Andisol soil (Table 1). The trend of C mineralization was better matched by the mineralization of N in the Andisol soil than in the Mollisol soil, since the latter showed a logarithmic trend instead of the linear one obtained from the Andisol soil (Fig. 1B and D). This represents a more active mineralization process in the Andisol soil, in contrast with the Mollisol soil where the process reached a peak, and then depleted around day 56. Nevertheless, N mineralization for both soils was more dynamic than C mineralization, showing a more active period of microbial activity within the first two weeks (Table 3).

Soil S mineralization showed a larger release of sulfate at the beginning of the experiment (first 7 days), and then decreased over the

Table 3. Carbon, nitrogen and sulphur mineralization rates of two cultivated soils with different levels of organic matter in different periods of a 112-day soil incubation experiment.
Tabla 3. Tasas de mineralización de carbono, nitrógeno y azufre en dos suelos cultivados con diferentes niveles de materia orgánica en distintos periodos de tiempo dentro de los 112 días de incubación del suelo.

Evaluated period (days)	Carbon mineralization (µg CO_2-C g soil^{-1} day^{-1})		Nitrogen mineralization (µg NH_4^+-N g soil^{-1} day^{-1})		Sulphur mineralization (µg SO_4^{-2}-S g soil^{-1} day^{-1})	
	Andisol	Mollisol	Andisol	Mollisol	Andisol	Mollisol
0-7	4.94 A (2.20)	3.86 B (1.70)	0.47 A (0.14)	0.52 A (0.12)	0.12 A (0.03)	0.07A (0.01)
7-14	4.87 A (1.91)	4.19 B (0.99)	0.51 A (0.09)	0.52 A (0.08)	0.08 AB (0.02)	0.06A (0.02)
14-28	5.98 A (2.53)	6.07 A (1.38)	0.21 B (0.03)	0.20 B (0.05)	0.04 BC (0.01)	0.02B (0.003)
28-56	3.79 A (1.23)	4.08 B (0.61)	0.12 C (0.01)	0.11 B (0.01)	0.01 CD (0.004)	0.01BC (0.004)
56-112	4.98 A (1.13)	3.88 B (0.87)	0.18 BC (0.01)	0.04 C (0.01)	0.003 D (0.002)	0.003 C (0.001)

Capital letters indicate statistical significance differences ($P \leq 0.05$) between mineralization rates evaluated within a soil. Standard deviation values of the variables are shown in brackets.

incubation period (Fig. 1E). Partial differences in S mineralization were statistically significant ($P \leq 0.05$) between the incubation periods evaluated. Consequently, the cumulative S mineralized over time showed a consistent difference between the soils, where the Andisol soil showed larger potential to release S than the Mollisol soil ($P \leq 0.05$) (Fig. 1F). The temporal rate of soil S mineralization showed a marked progressive decline during the incubation period (Table 3). In particular, the S mineralization rate dropped consistently after 14 days of soil incubation. The pattern of S mineralization was coincident with that of N mineralization of the Mollisol soil.

Specific parameters related to N organic release, such as urease activity and potentially available N (PAN) showed no statistical differences ($P \geqq 0.05$) between the Andisol and Mollisol soils (Table 4). The urease activity was greater than that reported in another study in an Andisol soil at the same site (Sanchez-Hernández et al., 2017), possibly due to the historical fertilizer application contrasted with the organic management. In turn, the PAN values observed in this study were within the ranges described for other soils (Baxter and Oliver, 2005).

In light of these results, the microbiological parameters analyzed showed no statistical differences ($P \leq 0.05$) between the soils. Statistical differences were found only in S mineralization. Due to the greater potential S response of the Mollisol, this soil was used to evaluate the effect of S application on N crop use, and its consequent effect on N uptake.

As available N and S were low in the Mollisol (Table 1), additional inputs were applied to improve the presence of these nutrients in the soil solution for plant uptake.

Effect of S application on crop development

Crop height over time was measured as a growth index to assess the response to N and S application (Table 5). The combined application of N and S showed better growth response ($P \leq 0.05$) than a single application of S or N. This was particularly observed when the crop was more developed (after day 80), as during the first month the differences between the treatments were less evident. Visually, the intensity of the green color in the leaves showed correspondence with the crop height registered per pot (data not shown). This would support the hypothesis of the role of S improving photosynthesis in the presence of N. The results also showed that after the initial growth, the sole application of S can improve crop height since no significant difference was found ($P \geq 0.05$) with respect to the N100S0 treatment. However, this pattern was not similar to that observed for the biomass production, as only the N application showed a significant effect

Table 4. Soil indicators of nitrogen availability for two cropped soils with different levels of soil organic matter.

Tabla 4. Indicadores de disponibilidad de nitrógeno en el suelo para los dos suelos cultivados con diferentes contenidos de materia orgánica.

	Potentially available nitrogen* (μg N-NH_4^+ g soil^{-1})	Urease activity* (μg N-NH_3^+ g soil^{-1} h^{-1})
Andisol	11.38	36.3
	(4.33)	(1.66)
Mollisol	15.89	37.79
	(7.55)	(3.80)

*No statistical significance (P ≧ 0.05) for these variables between soils.

Table 5. Plant height (*Lolium multiflorum* L.) evaluated through a growing season of sixteen weeks under the effect of sulfur (rates of 0 and 30 kg ha^{-1}) and nitrogen (rates of 0 and 100 kg ha^{-1}) application to the soil in a pot experiment.

Tabla 5. Altura de las plantas (*Lolium multiflorum* L.) evaluada durante una temporada de cultivo de dieciseis semanas bajo el efecto de aplicación de azufre (dosis de 0 y 30 kg ha^{-1}) y nitrógeno (dosis de 0 y 100 kg ha^{-1}) al suelo en un ensayo en macetas.

Days after sowing	N0S0	N0S30	N100S0	N100S30
16	1.02 B	1.16 AB	1.14 AB	1.29 A
	(0.09)	(0.10)	(0.12)	(0.02)
22	3.45 B	4.02 AB	3.67 AB	4.32 A
	(0.28)	(0.42)	(0.21)	(0.26)
30	5.51 B	6.07 B	6.00 B	7.17 A
	(0.18)	(0.29)	(0.35)	(0.12)
37	6.20 B	7.10 AB	7.29 AB	7.82 A
	(0.50)	(0.47)	(0.40)	(0.31)
51	7.20 C	8.40 B	8.32 B	9.51 A
	(0.23)	(0.25)	(0.28)	(0.23)
58	7.50 C	9.00 B	8.90 BC	10.90 A
	(0.269	(0.61)	(0.75)	(0.42)
65	8.80 C	10.00 BC	10.20 B	12.60 A
	(0.28)	(0.67)	(0.69)	(0.19)
71	11.00 B	11.80 AB	12.10 AB	14.00 A
	(0.17)	(0.44)	(0,84)	(0.20)
80	15.20 B	15.90 B	15.70 B	19.80 A
	(0.10)	(0.40)	(0.06)	(0.51)
87	17.80 B	18.50 B	18.20 B	21.20 A
	(0.28)	(0.43)	(0.42)	(0.50)
93	20.70 B	20.90 B	20.80 B	23.70 A
	(0.36)	(0.26)	(0.50)	(0.36)
99	24.70 B	25.10 B	25.10 B	28.00 A
	(0.35)	(0.51)	(0.40)	(1.48)
109	27.60 B	27.20 B	21.20 B	30.30 A
	(0.35)	(0.40)	(0.15)	(1.79)

Capital letters indicate statistical significance differences (P ≤ 0.05) between soil sulphur and nitrogen application treatments at each evaluation during a growing season.

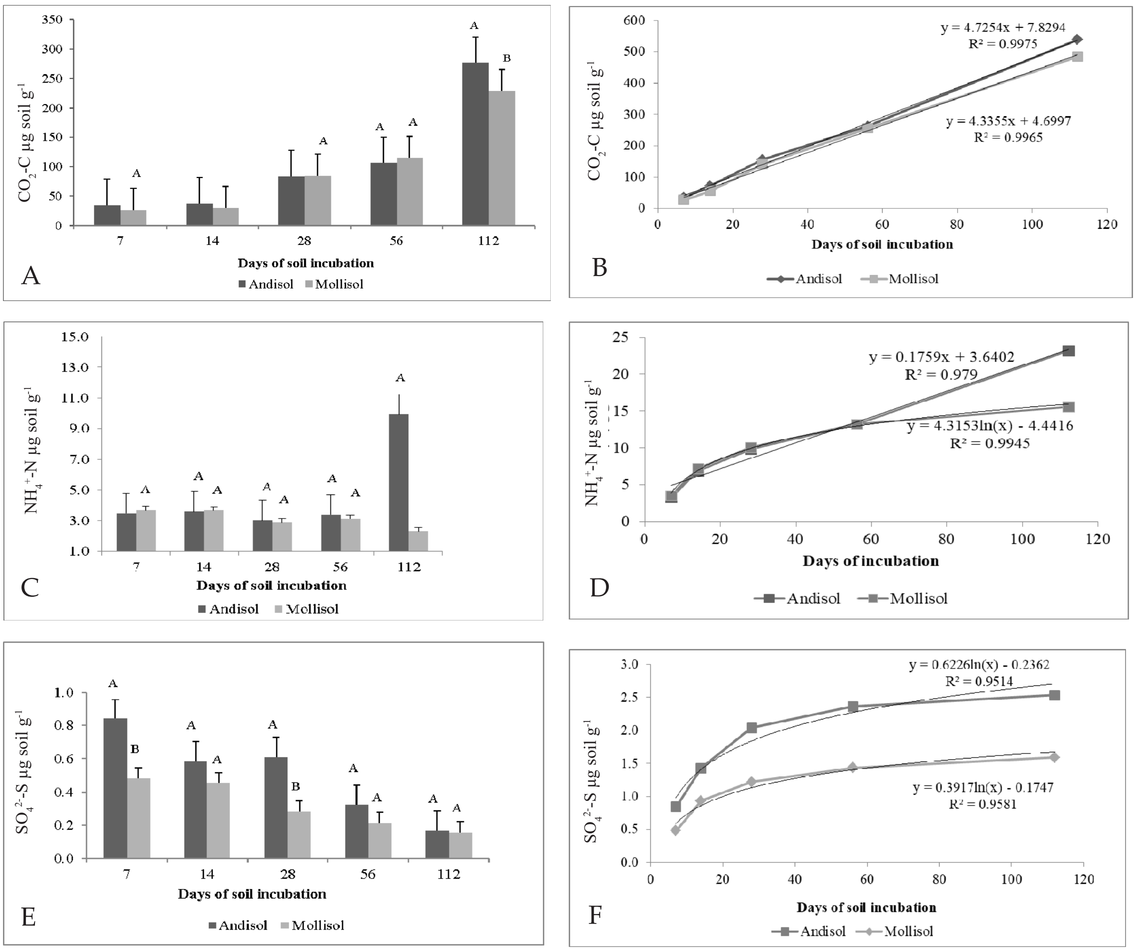

Fig. 1. Partial (A, C, E) and cumulative (B, D, F) mineralization of carbon, nitrogen and sulfur from Andisol and Mollisol soils.

Fig. 1. Mineralización parcial (A, C, E) y acumulativa de carbono, nitrógeno y azufre para los suelos Andisol y Mollisol.

on dry matter yield ($P \leq 0.05$) (Table 6). This is, the dry matter increment from the control treatment (N0S0) to N100S0 was 69%, and 74% from the control treatment with 30 kg S ha^{-1} (N0S30) to the combined top N and S application treatment (N100S30) (Table 6). In turn, the application of S did not show statistical significance ($P \geq 0.05$) within the treatments with no N application or N100 treatment. However, dry matter production increased by 8 and 23% under limited N and N100 application, respectively (Table 6).

The dry matter obtained from the root system evaluated at the end of the assay showed a similar trend to that observed for the foliage dry matter (Table 6). In particular, the combination of N and S resulted in higher plant root growth (over 18%, $P \leq 0.05$), but lower than that observed in the control and single nutrient treatments (Table 6).

Total N content in the foliage ranged from 1.39% to 2.63%, and as well as the dry matter production, showed only the effect of the N application (Table 6). The rate of 30 kg ha^{-1} of S did not improve the quality of the crop under the conditions of this study, which differs from the working hypothesis. However, essential amino acids containing S, like methionine and cysteine were not measured. S is part of their structure, particularly in the thiol group (cysteine), which has a key role at all levels of biochemical systems (Wirtz and Droux, 2005). Plants are exclusively the only organisms that can take up inorganic S (as sulfate), reduce it to the thiol group, and then to essential amino acids (Wirtz and Droux, 2005). Therefore, human beings are dependent on vegetable consumption to incorporate these essential amino acids into the diet. The

Table 6. Crop yield, N foliage concentration, and root growth in *Lolium multiflorum* L. grown in a pot experiment for sixteen weeks under sulfur (rates of 0 and 30 kg ha^{-1}) and nitrogen (rates of 0 and 100 kg ha^{-1}) application.

Tabla 6. Rendimiento del cultivo, concentración de N foliar y crecimiento de raíces del cultivo de *Lolium multiflorum* L. en un ensayo en macetas por dieciseis semanas con aplicación de azufre (dosis de 0 y 30 kg ha^{-1}) y nitrógeno (dosis de 0 y 100 kg ha^{-1}).

	Biomass			Root system		
	Dry matter (g pot^{-1})	N concentration (%)	Dry matter (g pot^{-1})	Area (cm^{-2})	Length (cm)	Volume (cm^{-3})
N0S0	1.53 B (0.10)	1.57 B (0.36)	2.01 B (0.19)	248.24 AB (21.37)	1867.96 AB (382.40)	12.17 AB (2.37)
N0S30	1.66 B (0.19)	1.39 B (0.28)	2.11 B (0.16)	267.21 A (45.10)	2258.05 A (178.50)	13.05 AB (1.83)
N100S0	4.92 A (1.00)	2.61 A (0.91)	9.99 A (2.43)	251.17 AB (26.63)	2171.10 A (340.10)	10.63 B (1.64)
N100S30	6.39 A (0.94)	2.63 A (0.99)	10.11 A (2.17)	222.13 B (38.52)	1676.90 B (304.10)	13.08 A (2.95)

Capital letters indicate statistical significance differences ($P \leq 0.05$) between crop variables harvested at the end of the growing season. Standard deviation values of the variables are shown in brackets.

measurement of these amino acids might have indicated whether S was taken up by the crop and improved the quality of the production.

The results indicate that N application had a greater impact on crop biomass yield than S application. This is probable explained by the very low initial N availability. The experiment might have benefited from the inclusion of a higher N application rate than the N100 applied in the assay. We acknowledge the number of treatments combining N and S doses used was low, but a greater number of treatments would have implied a larger experimental assay, being more difficult to manage. The treatments selected for this study were thought of as an initial starting point of the research, using N and S rates that could represent an economic alternative for crop fertilization. However, the suggested rates of S application might have to be revised due to the higher nutrient requirements from improved current crop varieties. Therefore, further research is recommended to evaluate additional rates of N and S, including the application of isotope techniques and measurement of the foliage area index, which would explain the lack of consistency between height growth and crop yield production.

Effect of S application on N recovery efficiency

The N content of plant biomass was used to calculate the N recovery efficiency at the end of the studied period as related to the available and added N in the soil (Table 7). Apart from the increase of over 25% in the N uptake recovery due to the S application, no statistical differences were found between the treatments ($P \geq 0.05$). This increase seems to follow a similar trend to that of the dry matter obtained from the S rate application of 30 kg ha^{-1} at the N fertilized pots, as N concentration was almost the same between the N100S0 and N100S30 treatments (Table 7). Therefore, an improvement in N uptake was not observed from the S application at the rates used here. However, it has been pointed out that the synergy between S and N works better when soil available N is high (Salvagiotti et al., 2009), which might indicate that the N rate applied in this study was under the dose required by the crop and, consequently, the S applied did not fulfill the expected function. This can be supported by the increased residual soil available S, which was around three-fold higher than the initial S status (Table 8), whilst residual N was almost two-fold higher than the initial soil available N. As discussed above, the analysis of essential S-containing amino acids in the produced biomass could provide a probe of S fate within the soil-plant system.

Table 7. Nitrogen recovery efficiency measured in *Lolium multiflorum* L. grown in a pot experiment for sixteen weeks under sulfur (rates of 0 and 30 kg ha^{-1}) and nitrogen (rates of 0 and 100 kg ha^{-1}) application.

Tabla 7. Eficiencia en la recuperación de N medida en *Lolium multiflorum* L. creciendo en un ensayo en macetas por dieciséis semanas con aplicación de azufre (dosis de 0 y 30 kg ha^{-1}) y nitrógeno (dosis de 0 y 100 kg ha^{-1}).

	Nitrogen recovery efficiency*
N100S0	230.5 (114.2)
N100S30	312.0 (114.5)

*No statistical differences were registered between the treatments ($P \geq 0.05$).

Table 8. Residual soil available nitrogen (N) and sulphur (S) contents after *Lolium multiflorum* L. was grown in a pot experiment for sixteen weeks under S and N application.

Tabla 8. Nitrógeno (N) y azufre (S) residual en el suelo después del cultivo de *Lolium multiflorum* L. en un ensayo en macetas por dieciséis semanas con aplicación de S y N.

	Soil available nitrogen (mg NO_3-N kg soil^{-1})	Soil available sulphur (mg SO_4^{-2}-S kg soil^{-1})
Initial soil content	5.28 B (0.30)	4.75 B (1.07)
N0S0	4.38 B (2.49)	2.64 B (0.71)
N0S30	5.33 B (2.95)	7.81 A (0.88)
N100S0	10.62 A (4.19)	2.76 B (0.51)
N100S30	10.79 A (6.80)	6.80 A (0.88)

Capital letters indicate statistical significance differences ($P \leq 0.05$) between soil available S and N during cropping season. Standard deviation values of the variables are shown in brackets.

CONCLUSION

The Andisol and Mollisol soils under study showed high contents of organic matter, but low N and S availability as well as a reduced capacity to supply mineral N and S to the soil solution. The natural supply from the Mollisol through N and S mineralization was lower than that observed in the Andisol. The response to a medium dose of S application in the Mollisol soil did not increase N recovery significantly ($P \geqq 0.05$) in the crop, but resulted in increased root system growth of the ryegrass. A wider combination of N and S application rates are recommended in order to prove the interaction of S with higher N availability for the crop.

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