Isotopic technique for tracing both reduced and oxidised forms of sulphur in a fertiliser

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Abstract

An isotopic technique was developed to trace both reduced and oxidised forms of sulphur in a fertiliser. Enriched ^{34}S (51.18 atom %) labelled-fertiliser was added to three different S responsive soils already labelled with a radioactive ^{35}S. The experimental design followed a randomised complete block design with 3 soils, two treatments (+S and –S) and 4 replicates. Canola plants were grown for 16 weeks under 20 °C/15 °C day/night temperatures. Harvested dry matter and seeds were analysed for total S by inductively coupled plasma optical emission spectrometry (ICP-OES) following a nitric acid digestion. Radioactivity of ^{35}S in the digests and atom % of ^{34}S in intact plant samples was measured on a liquid scintillation beta counter and a continuous flow isotope ratio mass spectrometry (CF-IRMS), respectively. From these measurements, the % of S derived from the fertiliser and specifically from ^{34}S° was calculated. There was a significant ($p < 0.05$) yield response to S in all three soils, with between 40 and 62% of the S in the plant coming from the added fertiliser. Over a 14 week period, < 10% of the elemental S in the fertiliser was taken up the plant. The combined stable and radioisotope technique has a potential for differentiating between plant uptake from sulfate-S and elemental S. This double labelling isotopic method can now be applied in the field to trace the uptake of SO_4-S and S°.

Key Words

Stable ^{34}S isotope, radioactive ^{35}S, CF-IRMS, atom %, specific activity.

Introduction

There is now an increasing recognition of S deficiency in agricultural soils, which has made the application of S-containing fertiliser a viable option. This is mainly due to the reduced atmospheric S deposition over the past few decades (Haneklaus *et al.* 2008). The increasing use of high analysis P fertiliser like mono- and di-ammonium phosphate (MAP and DAP), with low S content, over single super phosphate (SSP) might have also exacerbated this deficiency over the years (Blair 2008). The need to supply S continuously throughout the cropping season has led to different formulations of S-containing fertilisers; some of which have more than one form of S, mainly as quick release sulphate-S and slow release elemental S.

Isotopic techniques offer a superior quantitation of nutrient uptake from fertiliser than any other estimation of plant uptake. Isotopic technique has been used in the past to assess the efficiency of S fertiliser containing only one form of S (Karltun 1994; Di *et al.* 2000; IAEA 2001). In some of these studies, isotopic dilution was used. This involves the labelling of labile S and biologically incorporated S pools with a radioisotope ^{35}S and calculating the amount of plant S derived from the added S fertiliser indirectly from the ratio of labelled and unlabelled S in the plant. In other studies, the fertiliser was labelled with a stable ^{34}S and S uptake by plants measured by tracing (Zhao *et al.* 2001). Clearly, either of these two methods is limited in assessing the efficiency of different forms of S applied to the soil in the same fertiliser.

We therefore combined radio-isotopic dilution and stable isotopic tracing methods in order to distinguish S uptake from both oxidised and reduced S forms in fertilisers containing both these species. The labile and biologically incorporated S in the soil can be labelled with a radioisotope to quantify the amount of S taken up by the plant that came from the fertiliser. If one form of the S in the fertiliser can be labelled with a stable isotope and its uptake traced directly in the plant, the amount of S in the plant from each S form in the fertiliser can thereby be estimated.

The objective of the study was therefore to develop an isotopic dilution/tracing method to assess the efficiency of a fertiliser containing two forms of sulphur.

Methods

Labelling of fertiliser with enriched ^{34}S

The experimental fertiliser granules were made by grinding mono-ammonium phosphate (MAP), ammonium sulphate (AS), and highly enriched elemental S (99.8 atom %) in a "puck-mill". The particle size of the elemental S, as revealed by scanning electron microscopy ranged between 20 and 200µm. The mixture was then steam-granulated using a pan granulator. The S analysis of the resulting granules was as follows: total S = 10.95%, SO_4-S = 5.4%, S° = 5.5% and ^{34}S° = 51.18 atom %.

Pot experiments

Sulphur responsive soils were collected from 3 locations in South Australia. The soils range from slightly acid to neutral in reaction. Physicochemical characteristics of the soil are listed in Table 1. The soils were moistened to 10% of their maximum water holding capacity (MWHC) and uniformly labelled with carrier-free $^{35}SO_4^{2-}$ at the rate of 3 MBq/kg soil. The labelled soils were then allowed to incubate for 2 weeks to allow for an equilibration of the radioisotope with the labile and mineralisable S pool in the soils. A randomised complete block design (RCBD) was used with 3 soil types, 2 fertiliser treatments (+ S and – S), and 4 replicates, giving a total of 24 pots. Approximately 2 kg of each soil was packed into each pot to a bulk density of 1300 kg $m^{-3}$and wetted to 60% MWHC.

Table 1. Physicochemical characteristics of the soils.

	Texture	pH	Organic C	NO_3-N	Sulfate-S	Colwell P	K	DTPA Zn	ECEC
			%	(-------------------	mg/kg	-------------------)			$cmol_c$/kg
Coonalpyn	Sandy Loam	5.4	0.7	12	4.2	45	84	0.89	2.2
Monarto	Loam	7	0.8	4	2.8	5	332	0.42	6.99
Wynarka	Sandy Loam	6.7	1	7	3.6	49	249	3.58	7.13
Approx. critical value					<7	<30	<40	<0.8	

The ^{34}S labelled fertiliser was added to the +S treatment at the rate equivalent to 15 kg S/ha (equivalent to the rate of 52 kg P/ha by the same fertiliser). The fertiliser was applied to simulate broadcast application with incorporation. Nitrogen, P, K and Zn were balanced in all the pots at an equivalent rate of 152, 25, 32, and 1 kg/ha, respectively. Four pre-germinated canola seeds (*Brassica napus* L. var ATR-Stubby) were transplanted into each pot and thinned to 2 plants per pot after 5 days. The plants were watered daily to replace transpirational losses and grown for 16 weeks under 20 °C/15 °C day/night temperatures. Upon harvesting, the above-ground part were divided into leaf+stem and pods and dried at 50° C for 1 week. The total yield (dry matter plus seed) was recorded for each pot.

Total plant S and specific radioactivity of ^{35}S

One gram of ground dry matter (shoot+leaves+pods) from each pot was digested in concentrated HNO_3, filtered and analysed for total S by ICP-OES. Two ml of the plant digest was thoroughly mixed with 10mL of the EcoScint A scintillant and the activity of the ^{35}S measured on a 1215 RackBeta II liquid scintillation counter (LSC) (LKB Wallac, Finland). In addition, 0.5 g of the seeds was digested in the same manner as the dry matter and analysed by ICP-OES and LSC.

Stable sulphur isotope ratio analysis

The plant samples are still undergoing further radioactive decay before they are sent for stable S isotope analysis. As a preliminary, plant samples from similar experiment on Wynarka soil without labelling of the soil with ^{35}S were analysed for stable S isotope ratio using a modification of the continuous flow isotope ratio mass spectrometry (CF-IRMS) method described by (Monaghan *et al.* 1999). Tin capsules containing reference or plant samples (≤ 8 mg) plus vanadium pentoxide (12 mg) catalyst were loaded into an automatic sampler, from where they were dropped, in sequence, into a furnace held at 1080 °C and combusted in a 25 ml pulse of pure oxygen. The combusted gases were then swept in a He stream (60 ml/min) over combustion catalysts (tungstic oxide/zirconium oxide) and through a reduction stage of high purity copper wires to produce SO_2, N_2, CO_2, and water. Water was removed using a Nafion™ membrane. Sulphur dioxide was resolved from N_2 and CO_2 on a packed Porapak^{TM} QS GC column at a temperature of 32 °C .The excess CO_2 that preceded the SO_2 peak was 'valve-dumped' before entering the IRMS. Analysis was based on monitoring of *m/z* 48, 49, and 50 of SO^+ produced from disintegration of SO_2 in the ion source.

The reference material used for sulphur isotope analysis was an in-house standard IA-R036 (barium sulphate, $\delta^{34}S_{V\text{-}CDT}$ = +20.74 ‰). IA-R036, and two other in-house standards; IA-R025 (barium sulphate, $\delta^{34}S_{V\text{-}CDT}$ = +8.53 ‰) and IA-R026 (silver sulphide, $\delta^{34}S_{V\text{-}CDT}$ = +3.96 ‰) were used for calibration and correction of the ^{18}O contribution to the SO^+ ion beam. All these standards have been calibrated and traceable to NBS-127 (barium sulphate, $\delta^{34}S_{CDT}$ = +20.3 ‰) and IAEA-S-1 (silver sulphide, $\delta^{34}S_{V\text{-}CDT}$ = -0.3 ‰).

Calculations
The efficiency of the fertiliser (containing both elemental S and sulphate –S), expressed as % S derived from fertiliser (% Sdff), was calculated according to Equation 1.

$\% \text{ Sdff} = [1- (SA_{+S}/SA_{CONTROL})] \times 100$ [1]

where SA is the plant specific activity of ^{35}S (kBq mg^{-1} S), calculated as the ratio of ^{35}S radioactivity in plant (kBq/g) and total S concentration in the plant (mg/g).

The efficiency of the labelled elemental S was expressed as % S in the plant derived from elemental sulphur in the fertiliser (% SdfS°, which was calculated as a ratio of atom % of ^{34}S excess over the background in plant and that of the fertiliser, according to Equation 2. The % SdfS° was calculated for plants harvested after 14 weeks in Wynarka soil only.

$\% \text{ SdfS}^{o} = (\text{atom } \% \ ^{34}S \text{ excess}_{PLANT}/\text{atom } \% \ ^{34}S \text{ excess}_{FERTILISER}) \times 100$ [2]

Statistical analysis of the total plant yield and % Sdff was performed using GenStat (Release 10.2) and differences among the treatments were identified using least significant difference (l.s.d) test at the 0.05 probability level.

Results and discussion

Plant response to sulphur
There was a significant yield response to S fertiliser in all the soils. The response was higher in the neutral soils than the slightly acid sandy soil (Figure 1). The S response was expected given the available S level in the soils, which was lower than the approximate critical level (Table 1). The measure of the efficiency of fertiliser added shows that between 40 and 62% of the plant S came from fertiliser (Figure 2). This suggests that approximately 38%, 50%, and 60% of the S in the plant came from the soil in the Coonalpyn, Monarto, and Wynarka soils, respectively. In the plant grown on Wynarka soil over a 14 week period, < 10% of the elemental S in the fertiliser was taken up by the plant. This indicates that most of the S in the plant that was derived from the fertiliser came from the sulphate-S in the fertiliser.

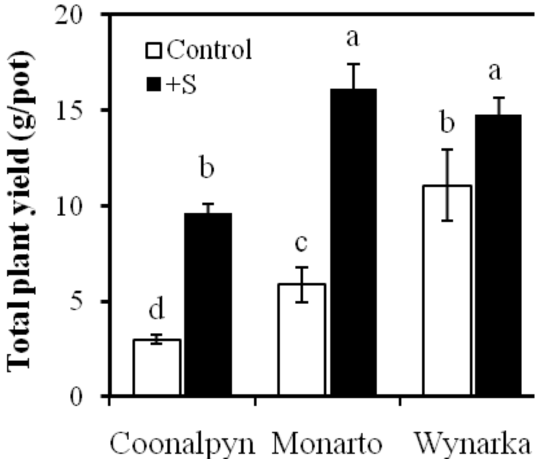

Figure 1. Canola yield response to S fertilisation. Mean with different letters are significantly different at 95% confidence level. l.s.d (5%) = 3.2g/pot

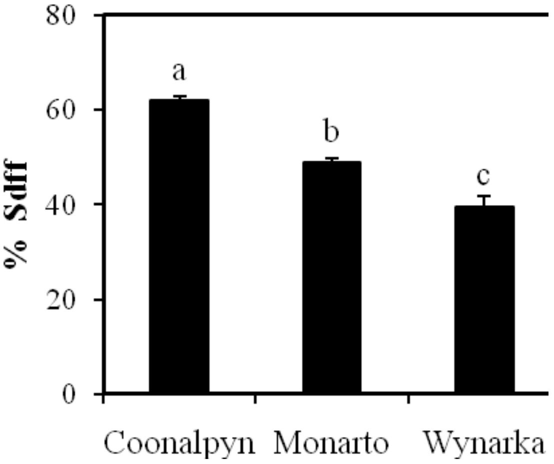

Figure 2. Proportion of plant S derived from fertiliser (%). Mean with different letters are significantly different at 95% confidence level. l.s.d (5%) = 8.5%.

By combining the enriched stable and radioisotope of S in a single experiment, there is a potential to quantify the amount of S taken up by the plant that originated from either the reduced or oxidized form of S in the fertiliser. Given the potential of the method presented in this paper to trace both SO_4-S and elemental S uptake by plants in a controlled environment, field plot experiments can now be carried out using the same procedure.

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