

# Study on the effect of Sulphur, Glucose, Nitrogen and plant residues on the Immobilization of Sulphate-S in Soil

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**Abstract**—In order to evaluate the relationship between the sulphur (S), glucose (G), nitrogen (N) and plant residues (st), sulphur immobilization and microbial transformation were monitored in five soil samples from 0-30 cm of Bastam farmers fields of Shahrood area following 11 treatments with different levels of Sulphur (S), glucose (G), N and plant residues (wheat straw) in a randomized block design with three replications and incubated over 20, 45 and 60 days, the immobilization of  $\text{SO}_4^{2-}\text{-S}$  presented as a percentage of that added, was inversely related to its addition rate. Additions of glucose and plant residues increased with the C-to-S ratio of the added amendments, irrespective of their origins (glucose and plant residues). In the presence of C sources (glucose or plant residues), N significantly increased the immobilization of  $\text{SO}_4^{2-}\text{-S}$ , whilst the effect of N was insignificant in the absence of a C amendment. In first few days the amounts of added  $\text{SO}_4^{2-}\text{-S}$  immobilized were linearly correlated with the amounts of added S recovered in the soil microbial biomass. With further incubation the proportions of immobilized  $\text{SO}_4^{2-}\text{-S}$  remaining as biomass-S decreased. Decrease in biomass-S was thought to be due to the conversion of biomass-S into soil organic-S. Glucose addition increased the immobilization (microbial utilization and incorporation into the soil organic matter) of native soil  $\text{SO}_4^{2-}\text{-S}$ . However, N addition enhance the mineralization of soil organic-S, increasing the concentration of  $\text{SO}_4^{2-}\text{-S}$  in soil

**Keywords**—Immobilization, microbial biomass, sulphur, nitrogen, glucose.

## I. INTRODUCTION

SULPHUR (S) immobilization in soil is the process through which mineral S is incorporated into soil organic compounds [10]. This process together with the mineralization of organic-S, regulates the accumulation and cycling of S in the soil and affects S availability to plants [6]. This immobilization is believed to be microbially mediated which includes the conversion of S into the microbial biomass (microbial utilization). However, the dynamics of the immobilization process and the mechanisms through which immobilization is associated with microbial transformations [2], (the utilization and turnover of S) remain poorly understood. Recent developments in methodologies for measuring soil microbial biomass-S [10] have facilitated progress towards the quantification of S transfer (rate and magnitude) between  $\text{SO}_4^{2-}\text{-S}$  microbial biomass-S and

organic-S pools in soil. [9] have shown that S immobilization in soils amended with plant residues (barley straw or rape leaves) is highly correlated with increases in microbial biomass-C. In a separate study using six soils with different properties, it was shown that the amounts of added  $\text{SO}_4^{2-}\text{-S}$  immobilized were comparable with those converted into the microbial biomass over the first few days [6], [7]. The subsequent incorporation of the immobilized  $\text{SO}_4^{2-}\text{-S}$  into the soil organic matter was through to depend on the turnover of the soil microbial biomass. Furthermore, this study demonstrated that the immobilization rates of  $\text{SO}_4^{2-}\text{-S}$  were influenced by soil properties such as clay and organic matter contents, the size of the microbial biomass and the available S. However, the extent to which these factors interact and affect the immobilization rates of S remained unknown.

This study showed the dynamics of the immobilization of  $\text{SO}_4^{2-}\text{-S}$  using amendments containing different rates of  $\text{SO}_4^{2-}\text{-S}$ , different form of carbon (glucose, straw) and N nutrient. The initial Immobilization rates of added  $\text{SO}_4^{2-}\text{-S}$  were correlated with the amounts converted to soil microbial biomass-S over first few days. Properties of decomposed biomass-S converted into the soil organic-S (incorporated into the soil organic matter) were determined on further incubation. Our objective was to establish the quantitative relationship between the immobilization and microbial transformation of S and to estimate the effects of factors such as the amounts of  $\text{SO}_4^{2-}\text{-S}$ , the supply of C and N on the immobilization and availability of  $\text{SO}_4^{2-}\text{-S}$  in soil. These data are essential in proving the hypothesis that the incorporation of  $\text{SO}_4^{2-}\text{-S}$  into the soil organic matter via the microbial biomass is the primary mechanism for the immobilization of inorganic-S in soils [6], [1].

## II. MATERIALS AND METHODS

Composite samples from 0-30 cm depth of the five farmer's field were collected, air dried, and passed through 2 mm sieve and kept for 10 days in 25°C and 100% humidity. Soil physical and chemical properties were done by standard methods and are presented in Table I.

TABLE I  
PHYSICAL AND CHEMICAL PROPERTIES OF THE SOILS

Site	Clay (%)	O.C (%)	pH	Ec (mS cm <sup>-1</sup> )	Total S mg kg <sup>-1</sup>	Available S mg kg <sup>-1</sup>
1	33	0.88	7.65	0.74	278	1.4
2	42	1.05	7.44	0.59	311	3.4
3	31	0.79	7.26	0.61	252	3.2
4	37	0.74	7.64	0.83	195	2.6
5	44	0.88	7.48	0.79	205	3.6

Sixty gram of dried soil were weighed into 125 ml jars and amended with S, N, glucose and plant residues as detailed in table 2. Controls were left unamended. Sulphur as K<sub>2</sub>SO<sub>4</sub> (S<sub>10</sub> and S<sub>25</sub> μ g g<sup>-1</sup>), N as KNO<sub>3</sub> and appropriate amounts of glucose and wheat straw were added to soil. For each treatment, a solution was prepared by dissolving the appropriate amounts of glucose, KNO<sub>3</sub> and K<sub>2</sub>SO<sub>4</sub> in 100 ml distilled water. An aliquot of the solution (4 ml) was mixed with each soil portion.

Before the addition of the solutions, plant residues (0.3 g) which had been dried (35° C) and ground were mixed with the soil portions as required. The control soil was treated with 4 ml distilled water to maintain equivalent moisture content to that of the amended soils. Following amendment, the soils were placed in 2.5 glass bottles, sealed and kept at 25° C and 100% humidity.

TABLE II  
DESCRIPTION OF THE TREATMENTS

Treatments	SO <sub>4</sub> <sup>2-</sup> -S (μg g <sup>-1</sup> soil)	NO <sub>3</sub> <sup>-</sup> -N (μg g <sup>-1</sup> soil)	Glucose (μg g <sup>-1</sup> soil)	Straw-C (μg g <sup>-1</sup> soil)
Control	---	---	---	---
S <sub>10</sub>	10	---	---	---
S <sub>25</sub>	25	---	---	---
S <sub>10</sub> +N	10	100	---	---
S <sub>25</sub> +N	25	100	---	---
S <sub>10</sub> +G	10	---	2500	---
S <sub>25</sub> +G	25	---	2500	---
S <sub>10</sub> +G+N	10	100	2500	---
S <sub>25</sub> +G+N	25	100	2500	---
S <sub>10</sub> +St+N	10	100	---	2500
S <sub>25</sub> +St+N	25	100	---	2500

The contents of SO<sub>4</sub><sup>2-</sup>-S and the microbial biomass-S were determined after 20, 45 and 60 days of incubation. At each sampling, one portion of soil from each treatment was removed and subdivided by weighing (6×10g) into centrifuge tubes (45 ml). Three of the sub-samples were fumigated for 24 h in CHCl<sub>3</sub> vapor [10]. The remaining three sub-samples were used as the controls and left unfumigated. All of the sub-samples were extracted in 10 mM CaCl<sub>2</sub> (20 ml) by shaking for 60 min at 400 rev min<sup>-1</sup> on an end-over-end shaker. Extracts were filtered through Whatman No. 42 filter paper and stored at -18°C prior to analysis.

Soil microbial biomass-S was measured by the procedures described by [10]. Briefly, total extractable-S was determined using [5] following conversion of organic-S in the extract into SO<sub>4</sub><sup>2-</sup>-S. This was done by digesting an aliquot of the extracts

(5 ml) in a 10 ml graduated glass tube for 24 h in a sand bath (160°C), using H<sub>2</sub>O<sub>2</sub> (AR grade, 30% v/v, 1.5 ml). Total biomass-S was calculated from the relationship B<sub>s</sub> = F<sub>s</sub>/K<sub>s</sub>, where F<sub>s</sub> is the difference between total extractable-S in the fumigated soil and that in the control soil, K<sub>s</sub> with the conversion factor (0.31), determined by [10]. Analysis of variance (ANOVA) was performed on all data sets. Data from all treatment were combined in correlation and regression analysis. The statistical package Minitab12 and excel were used.

### III. RESULTS AND DISCUSSION

#### Immobilization of added sulphate-S

The amounts of added SO<sub>4</sub><sup>2-</sup>-S recovered from all of the treatments using 10 mM CaCl<sub>2</sub> decreased over 60 days incubation (Fig. 1). These decreased were similar in that they were initially rapid but became slow with extended incubation. This agrees with the findings of [6] who measured the immobilization of added SO<sub>4</sub><sup>2-</sup>-S in a number of soils with different properties. In this study results showed that decreases in the recovery of SO<sub>4</sub><sup>2-</sup>-S were greater in those soils receiving larger additions of amendments (S<sub>25</sub>, S<sub>25</sub> + G and S<sub>25</sub> + G + N treatments, compared with S<sub>10</sub>, S<sub>10</sub>+G and S<sub>10</sub>+G +N treatments; Table II). This suggests that the amount of SO<sub>4</sub><sup>2-</sup>-S immobilized (converted into soil microbial biomass-S or incorporated into soil organic matter) in soil was, as expected, positively correlated with the addition rate. However, the extent to which SO<sub>4</sub><sup>2-</sup>-S was immobilized, as a percentage of addition, was inversely correlated to the addition rate. This is indicated clearly by the fact that the percentage of SO<sub>4</sub><sup>2-</sup>-S immobilized was smaller in those soils receiving the larger additions of SO<sub>4</sub><sup>2-</sup>-S.

In this experiment treatments with the addition of glucose markedly increased the immobilization of SO<sub>4</sub><sup>2-</sup>-S, particularly over the first 10 days of incubation. For example, in treatment S<sub>10</sub> + G + N the recoveries of SO<sub>4</sub><sup>2-</sup>-S in first 10 days was 50%, and by the end of incubation immobilization had decreased to over 35%.

In contrast, in those treatments receiving no additional C source (S<sub>10</sub>+S<sub>10</sub>+N), the immobilization of SO<sub>4</sub><sup>2-</sup>-S was less than 15% throughout the incubation. These results were expected, as it has been shown that the addition of labial substrate such as glucose can results in a rapid increase in microbial biomass which requires mores nutrient from the soil [4], [9], [6].

Based on the comparison of treatments S<sub>10</sub> and S<sub>10</sub> + N, and S<sub>25</sub> and S<sub>25</sub> + N, the addition of N without added C was unlikely to change significantly the immobilization of SO<sub>4</sub><sup>2-</sup>-S (Fig.2). However, the combination of N with glucose was shown to have a positive effect on S immobilization in soil. As shown in Fig. 2, in the treatments of S<sub>10</sub> + G + N and S<sub>25</sub> + G + N, the immobilization of SO<sub>4</sub><sup>2-</sup>-S increased by 12- 15% (as a percentage of the addition rates) by 20 days of incubation, when compared with treatments S<sub>10</sub> + G and S<sub>25</sub> + G. Thus, the supply of N can limit S immobilization in soil, particularly during rapid growth of the microbial biomass, as found following glucose addition [8].

Additions of straw residues ( $S_{25} + St + N$ ) increased the immobilization of  $SO_4^{2-}$ -S, compared with the treatment  $S_{25} + N$  which provide an equivalent amount of  $SO_4^{2-}$ -S and N but contained no residue amendment (Fig. 2). This was presumably a result of the rapid growth of the soil microbial biomass following the addition of plant residues [7], [1], [9]. However, the effect of plant residues on the immobilization of  $SO_4^{2-}$ -S was much smaller than that of glucose, since the amounts of  $SO_4^{2-}$ -S immobilized in treatments  $S_{25} + St + N$  were apparently less than those found in treatment  $S_{25} + G + N$ . Straw is considerably less labile and, at the same addition rate, might be expected to produce smaller increase in soil microbial biomass and S immobilization than either glucose or the straw residues [7], [9]. Previous studies by researchers have shown that the incorporation of plant residues with narrow C-to-s ratio (<200:1) can increase the contents of  $SO_4^{2-}$ -S in the soil, whereas the incorporation of those plant residues which have wide C-to-S ratios (<400:1) may result in a net immobilization of soil inorganic-S [7], [9]. It can be conclude that there are close relationships between the immobilization of  $SO_4^{2-}$ -S and the microbial utilization and turnover of S. The data support the hypothesis that primary mechanism by which  $SO_4^{2-}$ -S is incorporated into the soil organic matter is via the microbial biomass [6]. Since immobilization is dependent upon microbial utilization and turnover, the extent to which available-S ( $SO_4^{2-}$ -S) can be immobilized is determined by both the amount of available-S and the availability of an utilizable C source.

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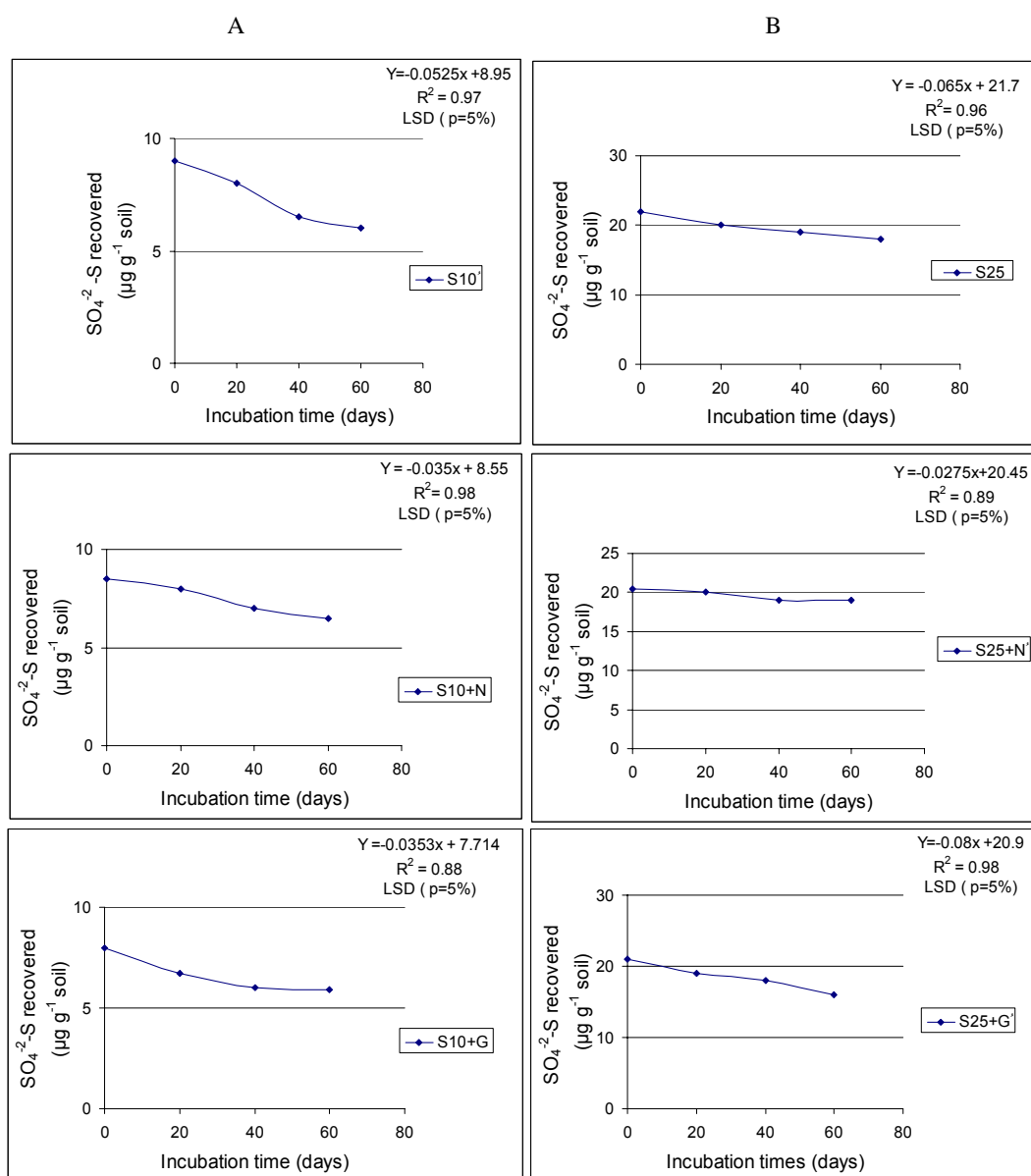


Fig. 1 Recovery of added SO<sub>4</sub><sup>2-</sup>-S (A) Treatments of S<sub>10</sub>, N and glucose (B) treatments of S<sub>25</sub>, N, and glucose. Details of the treatments are described in Table II

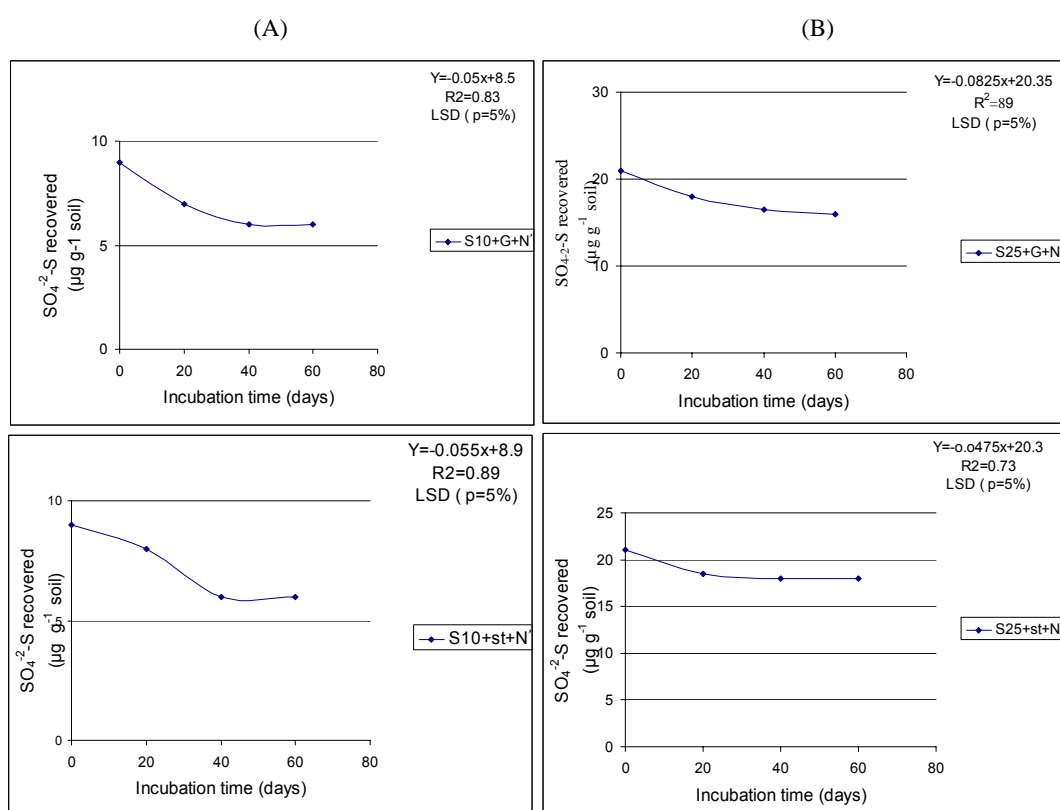


Fig. 2: Recovery of added SO<sub>4</sub><sup>2-</sup>-S (A) S<sub>10</sub> + G + N and S<sub>10</sub> St + N treatments (B) S<sub>25</sub> + G + N and S<sub>25</sub> + St + N treatments. Details of the treatments are described in Table II