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Multivariate factor analysis of sulfur oxidation and rhodanese activity in soils

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Abstract. The role of rhodanese as an intermediate catalyst in the oxidation of elemental S (S°) is not well understood. This study investigated the effect of 26 soil properties and steam sterilization in relation to S° oxidation and rhodanese activity in 33 soils (27 Oregon soils and six Chinese soils). S° oxidation potential was determined by incubating (7 d at 23 °C) soil amended with 500 mg S° kg^{-1} soil and measuring the SO_4 released. Both total S° oxidation (TSO) and rhodanese activity varied widely among the 33 soils, ranging from 0 to 143 mg SO_4-S kg^{-1} soil 7 d^{-1} and 22 to 2109 nmoles SCN^- g^{-1} soil h^{-1}, respectively. S° oxidation but not rhodanese activity had a significant positive correlation with soil pH. In sterile soils, chemical S° oxidation (CSO) averaged 3% of the total S° oxidation and apparent rhodanese activity averaged 11% of the total rhodanese activity. S° oxidation was not significantly correlated with rhodanese activity. However, development of stepwise regression models predicting S° oxidation revealed that rhodanese activity was an important explanatory variable in predicting biological S° oxidation (TSO minus CSO). Also, microbial biomass C was found to be an important parameter in models for both S° oxidation and rhodanese activity. Investigations of the effect of acidification during S° oxidation showed that biological S° oxidation was negatively correlated with S° oxidation-induced-pH-change for soils with pH ≤ 6 but no such significant relationship was found on soils with pH > 6. This suggested that extreme acidity may inhibit S° oxidation but not rhodanese activity.

Introduction

Reduced inorganic S compounds that are found or produced in the biosphere include sulfides (S^{2-}), elemental S (S°), thiosulfate ($S_2O_3^{2-}$), tetrathionate ($S_4O_6^{2-}$) and sulfite (SO_3^{2-}). Biotic and abiotic oxidation of these compounds is important in S cycling and in geochemical weathering of primary minerals. In the case of S° and thiosulfate, the oxidation reaction has important practical implications as these are routinely used as

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S fertilizers in agroecosystems. An enzyme involved in S oxidation is rhodanese, which catalyzes an intermediate reaction ($S_2O_3^{2-}$ to SO_3^{2-}) during oxidation of S°. Rhodanese is widespread in the biological world from man to bacteria to plants (Westley 1973).

Detection of rhodanese activity in soils (Tabatabai & Singh 1976) suggests that it has a role in S° oxidation, but studies of rhodanese activity in a limited number of soils have given conflicting results in relation to S° oxidation (Wainwright 1984; Ray et al. 1985). Preliminary evidence by Deng & Dick (1990) suggested that there was no consistent relationship between rates of S° oxidation and rhodanese activity among the limited number of soils tested. Although temperature and soil water potential have a strong influence on S° oxidation (Nor & Tabatabai 1977; Janzen & Bettany 1987a), results from Deng & Dick (1990) indicated that S° oxidation and rhodanese activity had differential responses to temperature and soil water potential.

S° oxidation and rhodanese activity have been detected in sterilized soils (Wiklander et al. 1950; Nor & Tabatabai 1977; Tabatabai & Singh 1976) suggesting that chemical S° oxidation (CSO) and chemical or apparent rhodanese activity (ARA) are possible. Little information is available on the relationship of CSO and ARA, and how these two factors relate to soil chemical properties, total S° oxidation potential and rhodanese activity. Lawrence & Germida (1988), in a study that did not measure rhodanese activity, showed significant correlations between S° oxidation and biomass C and concluded that the size and activity of the microbial biomass determined the rate of S° oxidation in agricultural soils. Soil pH has been reported as an important factor in S° oxidation (Vitolins & Swaby 1969; Nor & Tabatabai 1977; Germida et al. 1985), but little information is available on the effect of the pH buffering capacity of soils relative to S° oxidation and rhodanese activity. This would be important because S° oxidation is an acidifying process (Rudolph 1922; Nevell & Wainwright 1987; Janzen & Bettany 1987b), and the relative change in pH may be important in affecting microbial activity, including the synthesis and efficiency of a soil enzyme such as rhodanese.

The objectives of this study were to :

- determine the effect of soil sterilization on S° oxidation and rhodanese activity in relation to soil properties;
- determine the effect of soil acidification induced by S° oxidation on rates of S° oxidation; and
- determine the relationship between S° oxidation and rhodanese activity as influenced by soil properties.

Materials and methods

Twenty-seven soil samples that represent major soil types in Oregon and six major soil types in Guizhou and Hainan province, People's Republic of China were selected to include a wide range of chemical and physical properties (Table 1). Soil samples (0—15 cm depth) were obtained from a composite of 15—20 soil cores for each soil type. They were passed through a 2-mm sieve in the field-moist condition and air-dried at room temperature for 48 h by spreading soils in a thin (1—2 mm) layer on clean paper in the laboratory.

Soil moisture content, pH, soil texture, total P, total S, total C and microbial biomass C were determined as outlined by Deng & Dick (1990). Inorganic P (HCl/NaOH extraction) and organic P (total P minus inorganic P) were determined by the method of Olson & Sommers (1982). Sulfate was extracted with $Ca(H_2PO_4)_2$ (500 μg P mL^{-1}) and determined

Table 1. Mean and range of soil properties of Oregon and Chinese soil.

Parameter	Oregon soils (n = 27)		Chinese soils (n = 6)	
	Mean	Range	Mean	Range
Clay[a]	197	30—600	61.7	380—820
Sand[a]	378	60—890	89	40—184
pH	6.4	4.6—10.4	5.7	4.0—7.33
Bulk density (g cm^3)	1.07	0.69—1.44	ND[c]	ND[c]
Total C[a]	21	4.3—125	39.7	8.0—65.0
Total N[a]	1.61	0.41—8.99	1.98	0.43—4.23
Organic P[a]	0.24	0.01—1.55	0.34	0.18—0.50
Inorganic P[a]	0.69	0.24—2.05	0.59	0.25—1.07
Total S[b]	221	56—921	566	453—700
C-bonded S[b]	21	1.0—153	5.57	2.03—14.82
Ester sulfate-S[b]	82	31—358	495	344—812
Sulfate-S[b]	27	1.4—289	123	22—294
Biomass C[b]	255	43—1138	ND[c]	ND
Al[b]	58	0—969	233	0—1349
Cu[b]	0.77	0.19—2.60	0.91	0.32—1.62
Zn[b]	1.02	0.26—9.68	1.24	0.24—2.25
Fe[b]	20	0.27—206	9.19	0.07—23.9
Mn[b]	15.3	0.9—41	14.0	3.8—26.4
B[b]	0.47	0.16—3.23	0.24	0.10—0.38
Cl[b]	46	0.51—516	11.9	3—38

[a] g kg^{-1} soil
[b] mg kg^{-1} soil
[c] ND, not determined

by ion chromatography (Dick & Tabatabai 1979). C-bonded S, hydriodic acid reducible sulfate (HI-S) and total S (alkaline oxidation) were determined by the methods described by Tabatabai (1982). Ester sulfate was determined by the difference between HI-S and extractable SO_4-S. Iron, Zn^{2+}, Cu^{2+} and Mn^{2+} were extracted by DTPA (Olson & Ellis 1982) and $A1^{3+}$ was extracted with 1 *M* KC1, and all these cations were determined on an atomic absorption spectrophotometer. Chloride was extracted with deionized water (1:10) that was filtered and analyzed by ion chromatography. Boron was extracted by mannitol-calcium chloride and determined colorimetrically by the azomethine-H method (Bingham 1982).

S° oxidation potentials were determined by amending 20 g soil with 500 mg S° kg^{-1} soil, adjusting soil moisture to 60% water holding capacity (WHC), and incubating for 7 d at 23 °C. After incubation, the stopper was removed and 50 mL water was added (soil:water ratio, 1:2.5), and mixed thoroughly with the soil. After 45 min the pH of the soil was measured by a glass electrode. An additional 50 mL of 1000 μg P mL^{-1} $Ca(H_2PO_4)_2$ solution was added to each container to provide a final concentration of 500 μg P mL^{-1} $Ca(H_2PO_4)_2$. The bottle was stoppered, shaken on a rotating shaker for 1 h, and the extractable SO_4-S was determined. To account for native levels of SO_4 a control for S° oxidation was determined by following the same procedure outlined above in the absence of S° and this value was subtracted from the SO_4 produced in the presence of S°. Rhodanese activity (thiosulfatecyanide sulfurtransferase, EC 2.8.1.1) in soils was determined by the method of Tabatabai & Singh (1976). Chemical S° oxidation (CSO) was determined on soil that had been autoclaved at 121 °C and −0.6 MPa, three times at 24-h intervals. The soils were autoclaved for 30 min the first day and for 15 min on day two and day three to minimize changes in chemical properties due to steam sterilization (Williams-Linera & Ewel 1984). After steam sterilization, the soils were treated with S° (500 mg S° kg^{-1} soil). The S° had been prepared as described by Deng & Dick (1990) and sterilized by fumigation with $CHC1_3$ (Parkinson & Paul 1982). The soil was adjusted to 60% WHC with sterile deionized water and then incubated for seven days followed by extraction and determination of SO_4 as outlined above for S° oxidation potential. Apparent rhodanese activity (ARA) was determined by autoclaving the soil for 1 hr at 121°C and −0.6 MPa and then assaying for rhodanese. All glassware and lab utensils used to determine CSO and ARA were steam sterilized and all experimental procedures were conducted in a sterile hood. To check for sterility of the soils and S° after steam sterilization, soil was shaken in LB broth for 48 h at 30°C and the LB agar was inoculated and incubated for 36 h at 30°. No growth was noted on the agar.

Regression analysis assumes the variables being correlated are normally distributed. All soil properties, rhodanese activity, S° oxidation rates, ARA and CSO were checked for normality and only soil bulk density was normally distributed. Most of these parameters were normally distributed after a log transformation with the remaining being normally distributed after a square root or inverse transformation. Prior to any correlation or regression each parameter was transformed appropriately so that its normally distributed population was used during the analysis.

Microbial biomass C was determined on field moist soil, but all the S° oxidation determinations and the other soil analyses are the average of the duplicate determinations on a moisture-free basis.

Results and discussion

Effect of steam sterilization on S° oxidation and rhodanese activity

Nor & Tabatabai (1977) found that the rates of S° oxidation in steam sterilized soils were slightly lower than those of air-dried soils. Since they did not incubate the sterilized soils under sterile conditions, chemical S° oxidation could not be verified. Consequently in our study sterile conditions were maintained and verified.

Determination of the extent and degree of total S° oxidation (TSO) and rhodanese activity in sterile soils was conducted on 27 Oregon soils and 6 Chinese soils (Table 2). Both S° oxidation and rhodanese activity were detected in most steam sterilized soils (Table 2). This was termed chemical S° oxidation (CSO) and apparent rhodanese activity (ARA), respectively. CSO ranged from 0 to 6.9 (avg. 1.2) mg SO_4-S kg^{-1} soil 7 d^{-1}, which was 0 to 15% (avg. 3%) of the total S° oxidation.

Apparent rhodanese activity ranged from 0 to 94 (avg. 24) nmoles SCN^- g^{-1} soil h^{-1}, which was 0 to 80% (avg. 11%) of the total rhodanese activity (TRA) (Table 2). Of the 33 soils, 23 had 10% or less of the total rhodanese activity associated with ARA, which is comparable to the results of Tabatabai & Singh (1976) who reported that five Iowa soils had 0.6 to 10% of the rhodanese activity associated with ARA. Chemical rhodanese activity or ARA may be due to the following reaction reported by Singleton & Smith (1988):

$$S_2O_3^{2-} + CN^- \xrightarrow{\text{Spontaneous}} SCN^- + SO_3^{2-} \qquad (1)$$

They demonstrated that half of the rhodanese activity assayed in pure culture of *Thiobacillus* was from ARA. The proportion of ARA to TRA,

Table 2. Total and chemcal S° oxidation, total and apparent rhodanese activity and pH change due to S° oxidation in 27 Oregon soils and six Chinese soils.

Soil	Total S° oxidation	Chemical S° oxidation	Total rhodanese activity	Apparent rhodanese activity	−ΔpH
	mg SO_4-S kg^{-1} soil 7 d^{-1}		nmoles SCN g^{-1} soil		
		Oregon soils			
Agency	17.3	0.35 (2)[a]	75	10 (14)[b]	0
Alicel	68.0	1.12 (2)	101	6 (6)	0.45
Amity	20.1	0.18 (1)	2109	34 (2)	0
Carney	19.3	2.76 (14)	354	87 (6)	0
Coker	19.9	1.65 (8)	480	35 (7)	0
Era	24.6	1.18 (5)	91	14 (16)	0
Fordney	13.7	0.35 (3)	106	3 (3)	0.31
Fort Rock a	22.5	1.25 (2)	49	0 (0)	0.13
Fort Rock b	14.0	0.72 (5)	48	0 (0)	0.21
Hoopal	40.6	0 (0)	63	24 (38)	0
Imbler	46.5	1.08 (2)	65	0 (0)	0.52
Jory	12.4	0.01 (0)	305	7 (2)	0
LaGrande	91.9	1.43 (1)	118	36 (31)	0
Madras	30.5	0 (0)	210	12 (6)	0.16
Medford	26.8	1.82 (0)	403	30 (7)	0
Metolius	19.3	0.35 (2)	126	13 (10)	0
Newberg	13.3	0.12 (1)	471	8 (2)	0.15
Northrup	11.0	8.54 (78)	24	20 (80)	0.37
Nyssa	31.0	0.57 (2)	228	23 (10)	0
Owysha	25.0	2.20 (9)	201	15 (8)	0
Quillayute	24.4	0 (0)	22	0 (0)	0
Quincy	51.3	1.52 (3)	35	7 (19)	1.55
Shano	44.6	2.25 (5)	60	12 (19)	0.74
Stanfield	102.5	2.77 (3)	25	—	0
Walla Walla-P	9.5	0.11 (1)	163	31 (19)	0
Walla Walla-W	30.1	0.69 (2)	110	0 (0)	0.61
Woodburn	39.1	0 (0)	393	8 (2)	0.62
		Chinese soils			
CH1	14.0	0 (0)	199	20 (10)	0
CH2	142.9	1.31 (1)	561	52 (9)	0.35
CH3	39.1	2.52 (6)	611	68 (11)	0.16
CH4	39.8	1.29 (3)	428	34 (8)	0
CH5	0.0	6.89	1661	94 (6)	0.10
CH6	53.7	0 (0)	97	73 (9)	0

[a] Number in parenthesis is percent CSO of the total S° oxidation.
[b] Number in parenthesis is percent ARA of the total rhodanese activity.

however, showed wide variation among soils. Tabatabai & Singh (1976) could not detect thiocyanate formation when thiosulfate and cyanide were incubated without soil. This indicates that the chemical reaction (1) may require the presence of catalysts that are present in the reaction media.

Because no direct relationship between S° oxidation and rhodanese activity was detected by Deng & Dick (1990), it might be possible that chemical reactions confounded the results. Thus to isolate the biological activity, biological S° oxidation (BSO) was estimated by subtracting CSO from TSO. Similarly, biological rhodanese activity (BRA) was estimated by subtracting ARA from total rhodanese activity (TRA). There was no correlation between BSO and BRA ($r = -0.03$) (Table 3). However, the ARA and TRA were significantly correlated, suggesting that soil properties conducive for ARA may also be conducive for biological rhodanese activity.

Among all soils CSO and ARA were not significantly correlated (Table 3); however, for soils with pH >6, CSO and ARA were significantly correlated ($r = 0.49$*). This provides evidence that the reaction that converts $S_2O_3^{2-}$ to SO_3^{2-} is a possible pathway during S° oxidation under sterile conditions with soil pH >6. Regressions of TSO with BSO or TRA with BRA showed strong correlations (Table 3), which suggests the importance of biological activity in relation to S° oxidation or rhodanese activity.

S° Oxidation and soil acidification

After a one week incubation, S° oxidation decreased soil pH from 0 to

Table 3. Simple correlation coefficients (r) for paired relationships of total rhodanese activity, apparent rhodanese activity, biological rhodanese activity, total S° oxidation, chemical S° oxidation, and biological S° oxidation in 27 Oregon and 6 Chinese soils.

	Total rhodanese activity	Apparent rhodanese activity	Biological rhodanese activity	Total S° oxiation	Chemical S° oxidation
Apparent RA	0.53***	—	—	—	—
BRA	0.98***	0.58***	—	—	—
Total S° oxidation	−0.17	0.14	−0.05	—	—
CSO	−0.08	0.30	−0.01	0.40*	—
BSO	−0.12	0.23	−0.03	0.97***	0.21

*, **, and ***, significant at P 0.05, 0.01 and 0.001, respectively.

−1.55 pH units (avg. −0.20) (Table 2). Twenty of the 33 soils tested showed a pH decrease of less than 0.1 pH unit. Five soils (Imbler, Quincy, Shano, Walla Walla-W and Woodburn) showed a pH change ranging from −0.5 to −1.55 pH unit. With longer incubation times, these soil pH decreases probably would be greater. For example, Woodburn had a pH decrease of −0.62, −1.02 and −1.05 at 7, 14 and 28 d, respectively (data not shown). These results are consistent with Nevell & Wainwright (1987) who found that soil pH decreased by 0.5 to 1.25 pH units after 35 d in soil amended with S°. Janzen & Bettany (1987b) found under field conditions that soil amended with 200 kg S° ha^{-1} decreased soil pH by 0.3 to 0.6 after two months and by 0.1 to 0.4 pH units after 16 months.

Results from our study indicate that for a majority of soils, soil acidification will be minimal following S° oxidation. The five soils showing significant pH decreases had fairly high rates of S° oxidation (>30.1 mg SO_4-S kg^{-1} soil 7 d^{-1}), high sand content (except for Woodburn which was dominated by a high silt content, 70%) and pH $\leqslant 7.4$ (Table 2). The strong effect of soil buffering capacity on soil pH change was further demonstrated by a significant negative correlation of soil pH change to soil clay content ($r = -0.40$*).

Soil pH change was negatively correlated to BSO in soils with pH $\leqslant 6$ ($r = 0.60$*). These results are consistent with a study by Chapman (1989), who reported pH changes of −0.4 to −0.9 pH units after 20 d incubation and a nonlinear relationship between S° oxidation and S° application rates. He attributed this nonlinear relationship to reduced biological activity due to acidification at high rates of S° application. The same nonlinearity between S° oxidation and S° application rates was found by Deng & Dick (1990) for some soils. The acidity produced during S° oxidation may create an unfavorable microenvironment for heterotrophic S oxidizers. Lawrence & Germida (1988) demonstrated that heterotrophs are mainly responsible for S° oxidation in agricultural soils, where they prefer neutral to alkaline conditions (Vitolins & Swaby 1969). For soils with pH >6 there was no significant correlation of BSO and pH change (data not shown). This would be expected on neutral to alkaline soils because S° oxidation would not cause the final pH value to be as low as in more acid soils, because the initial pH is higher and alkaline soils would be highly buffered against pH decreases.

Relationship of S° oxidation, and rhodanese activity to soil properties

Among the soil properties tested, soil pH is an important parameter in relation to S° oxidation (Fig. 1). S° oxidation was higher in alkaline soils

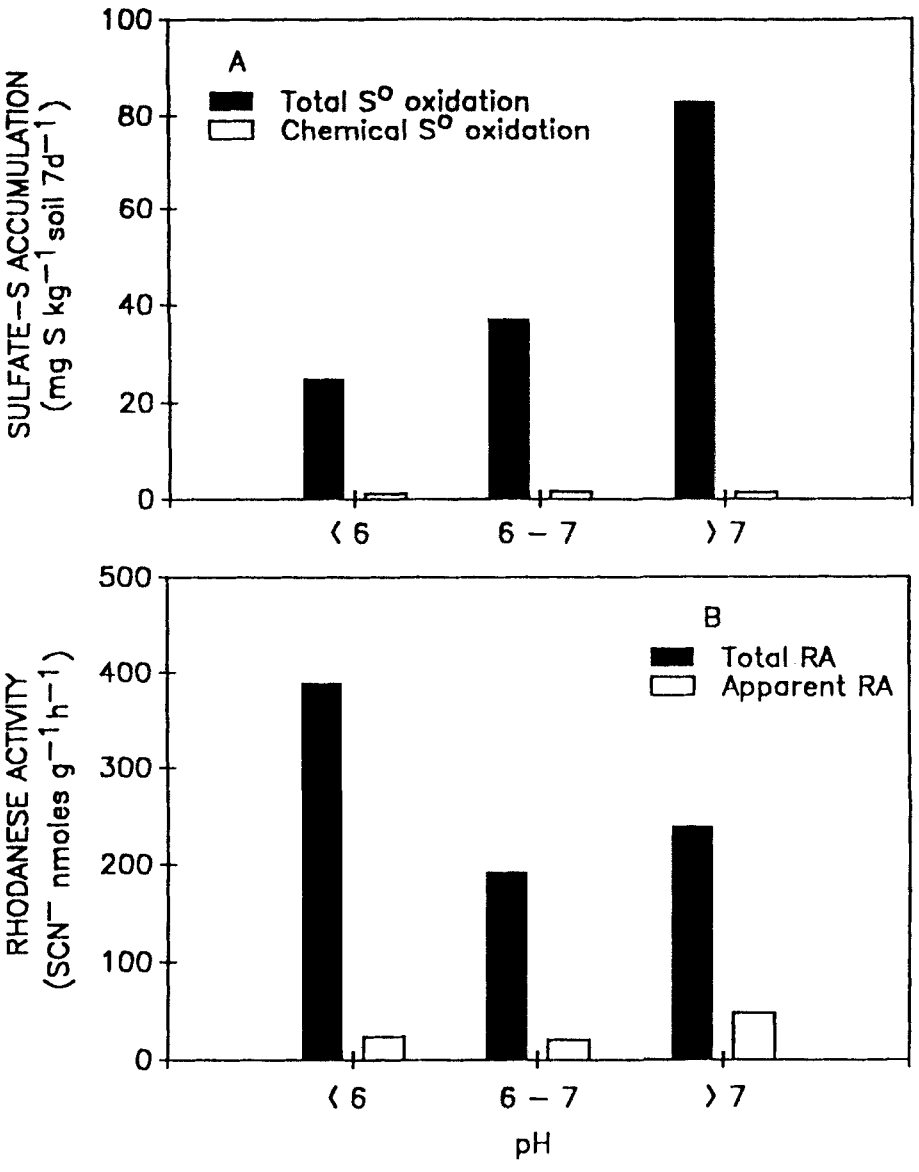

Fig. 1. Mean of total S° oxidation, chemical S° oxidation, rhodanese activity and apparent rhodanese activity for soils at pH ≤ 6, pH 6—7 and pH > 7.

than acid soils, but CSO was low in all the soils regardless of soil pH (Fig. 1A). The correlation coefficient of TSO and soil pH was 0.65*** (Table 4).

Total rhodanese activity showed great variation among soils with

Table 4. Simple correlation coefficients[a] (r) for paired relationships between rates of biological (BSO), chemical (CSO) and total (TSO) S° oxidation, and biological (BRA), apparent (ARA) and total (TRA) rhodanese activity with selected soil properties.

	Clay	Sand	pH	SO_4	Cu	B	Al	Mn	Cl	Zn	BD[c]
BSO	NS[b]	NS	0.50	0.51	−0.58	NS	NS	NS	NS	NS	NS
CSO	NS	NS	0.58	NS	NS	0.59	−0.62	NS	NS	0.40	0.42
TSO	NS	NS	0.65	NS	−0.42	0.47	−0.44	NS	0.35	NS	NS
BRA	0.48	−0.48	NS	NS	0.57	−0.41	NS	0.48	−0.39	NS	NS
ARA	0.68	−0.55	NS	−0.42	0.48	NS	NS	NS	NS	NS	NS
TRA	0.58	−0.55	NS	NS	0.59	−0.42	NS	0.44	−0.42	NS	NS

[a] All correlation coefficients reported were significant at $P < 0.05$.
[b] NS, not significant at $P < 0.05$.
[c] BD, bulk density.

standard deviations of 618, 187 and 216 nmoles SCN^- g^{-1} soil h^{-1} for soils with pH $\leqslant 6$, >6—7, and >7, respectively. This high variation at pH $\leqslant 6$ was due to a few soils with extremely high rhodanese activity (e.g. Amity). Total rhodanese activity tended to be higher in acid soils than in alkaline soils ($r = -0.30$*) (Fig. 1B). ARA was much lower than TRA in all the soils, but it tended to be higher in alkaline soils than in neutral or acid soils (Fig. 1B).

These results of increasing S° oxidation with increasing soil pH are consistent with other studies (Vitolins & Swaby 1969; Nor & Tabatabai 1977; Germida et al. 1985; Janzen & Bettany 1987b). This may be due to:

— S° oxidizers growing better in a more favorable pH environment; and
— decreases in pH following S° oxidation that may further inhibit S° oxidizers growth in acid soils as shown in the section on S° oxidation and soil acidification.

Conversely, organisms that synthesize rhodanese activity seem to be favored in an acid environment (Fig. 1B). The differential response of S° oxidation and rhodanese activity to soil pH is also consistent with results obtained by Deng & Dick (1990) who demonstrated that there may be several path-ways or enzyme systems involved in S° oxidation and rhodanese activity.

Many soil chemical and biological properties are related to soil pH. Thus the effect of soil pH on S° oxidation and rhodanese activity may be indirect by its effect on other soil properties. Among the 25 soil properties, 11 showed significant correlations to rhodanese activity or S° oxidation (Table 4). Clay content was only correlated to BRA, ARA and

TRA and not to any of the S° oxidation reactions. This relationship would be expected for rhodanese activity because rhodanese would partially exist as an extracellular enzyme and clay is important in complexing and protecting soil exoenzymes. Sulfate showed a positive correlation with BSO, but a negative correlation with ARA. The latter relationship is consistent with Deng & Dick (1990), who demonstrated that SO_4 repressed rhodanese activity. Copper can catalyze the chemical conversion of $S_2O_3^{2-}$ to SO_3^{2-} (Tabatabai & Singh 1976), and it was positively correlated to BRA, ARA and TRA, suggesting Cu++ plays a role in rhodanese mediated reactions in soils. The negative correlation of rhodanese activity and B (Table 4) is consistent with the results of Singh & Tabatabai (1978), who also found that inclusion of B in the assay inhibited rhodanese activity during the assay procedure. The negative correlation of rhodanese activity and Cl^- is consistent with a study by Roy & Trudinger (1970), who reported that Cl^- inhibited mammalian rhodanese activity. The inhibition has been explained, in part, by an increase in the ionic strength of the medium (Mintel & Westley, 1966). Conversely, a study by Singh & Tabatabai (1978) demonstrated that Cl^- activated rhodanese in soils when it was added to the rhodanese assay.

None of the soil parameters that might be potential indexes of microbial activity such as biomass C, total N and C, etc. showed significant relationships with either S° oxidation or rhodanese activity. However, because S° oxidation is the result of complex interactions of various factors, simple correlations are likely to be of limited use in establishing relationships of rhodanese activity and S° oxidation in relation to soil properties. Furthermore, the simple correlations only accounted for 12 to 42% of the variation for any given correlation. Consequently, multiple regression models were developed to predict rates of reaction for rhodanese activity and S° oxidation and to determine which soil factors are important in these reactions. In the case of S° oxidation, rhodanese activity was included in the model development to determine whether it is an explanatory variable.

Multiple-regression models were fitted by using the SAS PROC STEPWISE subprogram (Barr et al. 1976) with all 26 soil properties being initially available to be included in the model. Models that maximized the R^2 value were developed that only included variables with partial regression coefficients with significant t-tests $P < 0.05$. Because a high degree of collinearity among regressors would cause the model estimates to be unstable and have high standard errors, collinearity diagnostics and variance inflation were applied to each model (Belsley et al. 1980). With the exception of Fe (which was not included in any model), none of the regressors that made a significant contribution to the models violated collinearity. A final investigation of how well the models

fit was performed by plotting residuals with predicted values and residuals with each predictor variable. All plots were randomly distributed indicating the models provided a satisfactory fit.

Table 5 shows the final regression models for rhodanese activity. Both BRA and TRA had the same regressors in each model which provides evidence that catalysis of $S_2O_3^{2-}$ to SO_3^{2-} is largely controlled by enzymatic rather than chemical catalysis. Whereas simple correlations (Table 4) showed no significant relationships of rhodanese activity with biological indices, multiple-regression models for BRA and TRA showed that microbial biomass C made a positive and significant contribution to the models. This inidcates that the rate of rhodanese activity is the result of complex interactions of several soil properties. As with simple correlation, Cu^{2+} was significant in all the rhodanese models. Boron made a significant positive contribution to ARA, and Mn made a positive contribution to BRA and TRA. These relations are contrary to the results of Singh & Tabatabai (1978) who found that direct additions of B and Mn (50 μmole g^{-1} soil) during the assay inhibited rhodanese activity in soils. Sand content had negative partial correlation coefficients with BRA and TRA which would be expected since soils high in sand would have lower clay and organic matter to complex and stabilize free rhodanese.

Table 5. Multiple regression equation[a], standard partial regression coefficients, and coefficients of determination for predicting biological (BSA), apparent (ARA), and total (TRA) rhodanese activity from soil properties.

Regression equation[b]	R^{2c}
BRA Y[d] = 5.12 − 1.43 (TN)[f] + 0.816 (Cu) − 0.924 (SA)[g] + 0.671 (BIOC)[h] + 0.118 (Mn)	0.83
(−1.53)[i] (0.831) (−0.683) (0.696) (0.021)	
ARA Y = 0.634 + 0.298 (pH) + 0.684 (Cu) + 0.186 (B) − 0.193 (SA) + 0.099 (SO_4)	0.57
(0.97) (0.533) (0.223) (−0.126) (0.046)	
TRA Y = 4.204 − 1.12 (TN) + 0.859 (Cu) + 0.665 (BIOC) − 0.802 (SA) + 0.083 (Mn)	0.84
(−1.20) (0.871) (0.687) (−0.593) (0.014)	

[a] To normalize the data for model development, all enzyme activities and regression variables had log transformations, except for Mn which had a square root transformation.

[b] All regression coefficients were significant at 0.05 P.

[c] All R^2 values were significant at 0.001 P.

[d] Y = nmoles SCN g^{-1} soil h^{-1}.

[e] TN, total N content.

[f] SA, percent sand.

[g] BIOC, microbial biomass C.

[h] Numbers in parentheses are standardized partial regression coefficients = $b_i\ S_i/S_y$; where b_i is the partial regression coefficient (regression of Y on X_i for fixed values of other Xs). S_i = standard deviation of b_i, S_y = standard deviation of Y.

Standardized partial regression coefficients can be used to rank or give the relative importance of each variable in its contribution to the regression model. For chemical catalysis of the rhodanese reaction (ARA), soil pH was the most important, and it had a positive correlation coefficient. In the case of BRA and TRA, total N was the most important variable. It is difficult to explain the negative correlation coefficient associated for total N. One would have expected this to be positive because biomass C had a positive partial regression coefficient, and normally soils higher in N would be expected to be higher in biological activity.

Multiple regression models are shown for S° oxidation in Table 6. BSO and TSO had very similar models whereas CSO had a completely different set of variables in its model. The similarity of BSO and TSO indicates that S° oxidation in soils is dominated by biologically mediated processes. Unlike simple correlations where biomass C did not show a significant correlation with TSO, in the multiple regression model biomass C makes a significant contribution. This is consistent with Lawrence & Germida (1988) who found a significant relationship of S° oxidation with biomass C and soil respiration. Previous work by Deng & Dick (1990) showed no direct relationship between rhodanese activity and S° oxidation in relation to the effects of air drying soil, and temperature and soil moisture content.

Table 6. Multiple regression equations[a], standardized partial regression coefficients, and coefficients of determination (R^2) for predicting biological (BSO), chemical (CSO), or total (TSO) S° oxidation rates from selected soil properties.

Regression equation[b]	R^{2c}
BSO $Y^d = -1.09 + 2.41$ (pH) $- 1.12$ (Cu) $+ 2.301$ (BIOC)[f] $+ 0.141$ (RA)[g] $+ 0.119$ (Mn)	0.79
(7.195)[h] (−1.321) (0.226) (0.089) (0.023)	
CSO $Y = 5.72 + 9.89$ (B) $- 3.04$ (Al) $- 2.41$ (SO_4)	0.69
(10.98) (−1.135) (−1.076)	
TSO $Y = -0.434 + 2.01$ (pH) $- 0.574$ (Cu) $+ 0.243$ (BIOC) $+ 0.084$ (Mn)	0.76
(5.99) (0.582) (0.180) (0.0160)	

[a] To normalize the data for model development, all enzyme activities and regression variables had log transformations, except for Mn which had a square root transformation.

[b] All partial regression coefficients were significant at 0.05 P.

[c] All R^2 values were significant at 0.001 P.

[d] Y = mg SO_4 kg^{-1} soil 7 d^{-1}.

[e] BIOC, microbial biomass C.

[f] RA, rhodanese activity.

[g] Numbers in parentheses are standardized partial regression coefficients = b_i S_i/S_y; where b_i is the partial regression coefficient (regression of Y on X_i for fixed values of other Xs). S_i = standard deviation of b_i, S_y = standard deviation of Y.

Also, as mentioned above there was no significant, simple correlation of S° oxidation with rhodanese activity. However, in the stepwise model, rhodanese activity was included as a potential variable, and in the case of BSO it made a contribution to the model. This provides evidence that rhodanese does play a role in S° oxidation, but there may be several S° oxidation pathways. The reaction rhodanese catalyzes may not be the rate limiting step in S° oxidation.

Whereas soil pH was not an important factor in rhodanese activity (Table 5), it was the most important factor for BSO and TSO with positive partial correlation coefficients which are consistent with simple correlations in Table 4.

Boron appears to be an activator of chemical S° oxidation but Al and SO_4 appears to inhibit CSO as evidenced by the signs of their respective partial correlation coefficients (Table 6). Excess SO_4 would inhibit the chemical catalysis of S° because it would shift the equilibrium of the reactions toward maintaining S° rather than forming the SO_4 product.

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