CHAPTER VI

EXPERIMENTS WITH RADIOACTIVE SULFUR

Abstract. Previous research suggested black layer in turfgrass soils was associated with accumulations of S^{-2}. A convenient way to study S^{-2} formation was reported to be with ^{35}S as a tracer. This technique was used to examine in-situ black layer from a 'Penncross' creeping bentgrass green. Injection of tracer as $^{35}SO_4$ directly into intact black layer soil micro-cores resulted in production of $H_2{}^{35}S$ and acid volatile $^{35}S^{-2}$. Injection of tracer into black layer soil placed in a reactor vessel resulted in $SO_4{}^{-2}$ being reduced at a rate of 1.5 nMols S cm^{-3} soil day^{-1}. Addition of $NO_3{}^-$ at 48 kg N ha^{-1} reduced the rate by 7 fold. Elemental ^{35}S was reduced at a rate of 6.5 nMols S cm^{-3} soil day^{-1}. This research suggested that active bacterial $SO_4{}^{-2}$ reduction occurred in black layer and that elemental S reduction probably also occurred. It also supported the hypothesis that S was involved in black layer formation. Further, it suggested that fertilizer $NO_3{}^-$ would reduce the rate at which black layer S^{-2} forms.

Black layer formation in turfgrass soils has been reported to result from dissimilatory $SO_4{}^{-2}$ reduction (2, 5, 11, 13). Radioactive ^{35}S has been used to study in-situ $SO_4{}^{-2}$ reduction processes, mostly in marine or marsh sediments (6, 8, 14, 16). Rates of $SO_4{}^{-2}$ reduction in sea-water and in bottom sediments of the Black Sea were estimated by Sorokin (16) using $^{35}SO_4{}^{-2}$. Reported rates ranged from 2.3 to 0.03 mg S^{-2} produced L^{-1}

sediment/water day^{-1}. Smith (14) reported that addition of both $^{35}SO_4^{-2}$ and ^{35}S to profundal surface sediments of a freshwater lake resulted in production of $H_2{}^{35}S$ with no lag time. Rates of $^{35}SO_4^{-2}$ reduction were reported to vary between 0.9 umols SO_4^{-2} reduced L^{-1} hr^{-1} at the in-situ [SO_4^{-2}] and 19.1 umols SO_4^{-2} reduced L^{-1} hr^{-1} at a 1 mM [SO_4^{-2}]. Rate of elemental ^{35}S reduction was reported to be 8.8 umols S L^{-1} day^{-1}. Smith (14) and Jorgensen (8) also reported using $^{35}SO_4^{-2}$ in research involving model sediment reactor systems. The objective of this research was to use $^{35}SO_4^{-2}$ and elemental ^{35}S to determine whether S cycling occurs in black layer.

MATERIALS AND METHODS

Sulfide Distillation Apparatus. A S^{-2} distillation and collection apparatus similar to the one described by Jorgensen (8) and Smith (14) was constructed. The apparatus consisted of a 200 ml screw top canning jar (Ball Glass) as a reaction vessel. The vessel was connected from the lid by glass line to a series of 20 ml scintillation vials containing 3 (or 5) mls of 2% $CdCl_2$ (i.e., S^{-2} traps). Vials were arranged so that the vessel atmosphere containing volatile S^{-2} could be continuously purged with O_2 free N_2 exhausted through each of the traps in sequence. The exhausted $H_2{}^{35}S$ (or acidified $AV^{35}S$) would then react with the $CdCl_2$ according to the following reaction:

$$H_2{}^{35}S + CdCl_2 \quad <------> \quad Cd^{35}S + 2\ HCl \quad pK = -27 \ (10).$$

The resulting $Cd^{35}S$ produced in each trap would then be quantified directly by liquid scintillation counting (14). There were 4 vials in the trapping train, which eventually exhausted into a 125 ml erlenmeyer flask containing 100 mls of 2% $CdCl_2$. Sample from the exhaust trap would then also be counted.

The jar lid was also attached to a Hungate type (7) gassing system equipped with a pressure regulator. The N_2 gassing line passed through a flask of boiled water amended with cysteine as reducing agent. This was done to humidify the gas and minimize vessel evaporation. The gas inlet port on the inside of the vessel lid was also equipped with a teflon needle arranged so that incoming gas would first bubble into vessel solution before being exhausted. In addition, the jar lid was equipped with a stoppered port to facilitate entry into the vessel by needle.

Sulfur Cycling in Black Layer. The objective of this experiment was to determine whether $^{35}SO_4^{-2}$ would be cycled into $H_2{}^{35}S$ directly in an existing black layer. Intact micro-cores of black layer soil from the 10 cm depth of a 'Penncross' creeping bentgrass (Agrostis palustris Huds.) green were collected for the experiment in December, 1989. Moisture content of the intact black layer soil was 10%. An air dried composite sample of the soil from the 0-15 cm depth in that green was 0.07% N and 0.8% C. The SO_4^{-2} concentration in air dried sediment core soil was between 22 and 45 ug SO_4^{-2} cm^{-3} soil (17) and was variable from core to core. Most probable number estimates (1) indicated an approximate concentration of between 3.3×10^3 and 7×10^5 sulfate-reducing bacteria per gram of black layer soil. The soil

was a modified sand (88.3% sand, 5.0% silt, and 6.7% clay). Thatch was 5 cm thick. The green was located at the Robert Hancock Turfgrass Research Center in east Lansing.

Micro-cores were constructed from 10 cc syringe cylinders (Becton-Dickenson) impaled horizontally into the black layer. Syringe cylinders containing 10 cc of soil were capped with rubber stoppers and transported to the laboratory for storage. Micro-cores were stored in an anaerobe jar with an atmosphere of 90% N_2 and 10% H_2 at ambient temperature in the dark until time for use. Black layer was visible in each core but appeared variable from core to core.

Triplicate micro-cores were then injected with 1/2 ml of an active carrier-free $Na_2{}^{35}SO_4$ solution diluted in 10^{-3} M Na_2SO_4. Further, the label was also added as 10^{-3} Na_2SO_4 in 10% Na_2MoO_4, or in 10% NaN_3. One half ml of each solution contained 0.3 uCi of $^{35}SO_4{}^{-2}$ (i.e., 11,000 dps or Bq). Cores were then incubated in an anaerobe jar at 23 C in the dark for 2 days. After incubation micro-core soil was placed in the reaction vessel. The vessel lid was attached, and the vessel was purged of air with N_2 for 1 min. Next, 20 mls of freshly boiled distilled water cooled to room temperature under N_2 was added to the vessel through the stoppered port in the lid, and the N_2 flow rate was adjusted to near 100 mls min-1. In this fashion any volatile $H_2{}^{35}S$ which had formed was distilled from the soil solution in the vessel and exhausted into the Cd traps for quantification. After 30 minutes of distillation traps were changed and 2 mls of anoxic 37% HCl was injected into the vessel. This liberated any acid volatile $^{35}S^{-2}$ ($AV^{35}S$) which was subsequently distilled for

30 min. For each trap 15 mls of LSC cocktail for aqueous solutions (RPI, Mt Prospect, IL) was added directly and the amount of radioactivity distilled into each trap was then determined by counting in a Beckman LS 8100 scintillation counter. Samples were corrected for quenching by the H# method.

Time Course Studies with a Reactor Vessel. The objective of this experiment was to document the time course of appearance of $H_2{}^{35}S$ and $AV^{35}S$. A subsequent objective was to determine the fate of the added ^{35}S label. Twenty cc of black layer soil was added to the reactor vessel. This soil was identical to that used in the preceding section. The lid was attached then the vessel was purged of air with N_2 for 1 min. Thirty mls of freshly boiled distilled, deionized water cooled to room temperature under N_2 was then added to the vessel by syringe. The N_2 flow rate through the trapping train was then adjusted to near 100 mls min^{-1}. Sediment in the vessel was allowed to equilibrate under the moving N_2 atmosphere for 3 days. This was done to allow for any anaerobic microbial growth which may have been limited by moisture or exposure to ambient atmosphere.

After the equilibration period fresh $CdCl_2$ traps were attached to the trapping train, then 0.3 uCi of carrier free $Na_2{}^{35}SO_4$ in deionized water was injected into the vessel. The gas flow rate was maintained at near 100 mls min^{-1}. Just prior to the injection of the label 5 mls of vessel soil solution was withdrawn by syringe for determination of $SO_4{}^{-2}$ concentration (17). Traps were changed each hour for the first three, then again at hours 6, 12, and finally at hour 24. At hour 24, 3 mls of 10% NaN_3 and 2 mls of 37% HCl were then injected into the

vessel to halt bacterial activity and to liberate AVS respectively. The vessel was purged until radioactivity in the exhaust decreased to near background. For each trap 15 mls of LSC cocktail was then added directly and the amount of radioactivity distilled into each trap was determined by counting in a Beckman LS 8100 scintillation counter. Samples were corrected for quenching by the H# method. In a subsequent experiment NO_3^- as KNO_3 was added concurrently with the $^{35}SO_4^{-2}$ at a level of 48 kg N ha^{-1}, then analyses were performed in an identical fashion.

Rates of SO_4^{-2} reduction were calculated according to the following equation:

$$([SO_4^{-2}])(a)(1.06) \;/\; (A)(V)(t) = nM\ S\ cm^{-3}\ day^{-1}$$

where $[SO_4^{-2}]$ is the sulfate concentration in nM cm^{-3}, a is the radioactivity of H_2S plus AVS, 1.06 is an isotope fractionation factor (used by both Jorgensen and Sorokin), A is the original amount of radioactivity added, V is the sediment volume in cm^{-3}, and t is the time of incubation in days (8).

After the trapping of volatile ^{35}S was complete the vessel soil was extracted with several portions of fresh water until radioactivity was near background. Soil was oven dried at 60 C for 24-48 hrs then re-extracted with several portions of fresh benzene again until radioactivity was near background. After oven drying a second time the soil was digested in alkaline NaOBr (18) and again extracted with water. Portions of all extracts (5 mls) were filtered to 0.2 um, then counted in a Beckman LS 8100 liquid scintillation counter. Fifteen mls of LSC mix for aqueous samples was added to water samples while 10 mls of LSC for

non-aqueous samples was added to the benzene. The extractions were performed to roughly determine how much of the added label remained as $^{35}SO_4^{-2}$, how much oxidized to ^{35}S, and how much was incorporated into an organic fraction.

Reduction of Elemental S. The objective of this experiment was to determine whether elemental S was reduced directly. Elemental ^{35}S (1 uCi mg^{-1}) was dissolved in benzene giving a solution with specific activity of roughly 13 uCi ml^{-1}. This was determined by liquid scintillation counting of 0.1 ml solution. An aliquot of this solution, 10x larger by volume than that counted, was transferred to a 125 ml serum bottle (Wheaton) and allowed to evaporate, leaving behind a residue of ^{35}S. Eighteen cm^{-3} of black layer soil from the 10 cm depth of a 'Penncross' creeping bentgrass golf green was then added to the bottle. This black layer soil was collected in May, 1990 and was 14% moisture. Next, 50 mls of freshly boiled distilled water cooled to room temperature under N_2 and amended with 3 mls of 2% cysteine was added. The bottle was thoroughly purged of air with N_2 then stoppered with butyl rubber and crimped with aluminum. The bottle was then attached to a modified trapping train similar to the one previously described. In this trapping train the bottle became the reaction vessel, and was attached to the train and Hungate apparatus with syringe needles and tygon tubing through the stopper. Fresh S^{-2} traps were attached and the N_2 flow rate was adjusted to near 100 mls min^{-1}. Traps were changed each hour for the first three, then at hours 6, 18 and 24. At hour 24, 3 mls of 10% NaN_3 and 2 mls of 37% HCl were added to the bottle to

halt bacterial activity and release AVS respectively. Bottle exhaust was then trapped until radioactivity was near background. Radioactivity in traps was determined as previously described.

In a supportive experiment, 200 g of soil from a 'Penncross' creeping bentgrass golf green contained in 125 ml serum bottles (Wheaton) was treated with cold S from either Na_2SO_4 or from 52% flowable S at a level of 48 kg S ha^{-1}. Experimental units were then water-logged with tap water so that no headspace existed, stoppered with butyl rubber and crimped with aluminum. Units were then incubated for 21 days at 30 C in the dark. After 21 days units were sampled for concentration of free H_2S and AVS with the method of Cord-Ruswich (4).

RESULTS AND DISCUSSION

Sulfur Cycling in Black Layer. Active ^{35}S cycling was evident when $^{35}SO_4^{-2}$ was injected into existing black layer (see Table 1.). After 2 days approximately 4.4% of the recovered label existed as free $H_2{}^{35}S$ while 95.6% of the recovered label existed as $AV^{35}S$. The trapped $^{35}S^{-2}$ represented nearly one third of the added label. It was assumed that the e^- donor was sloughed turfgrass root mass or other organic debris.

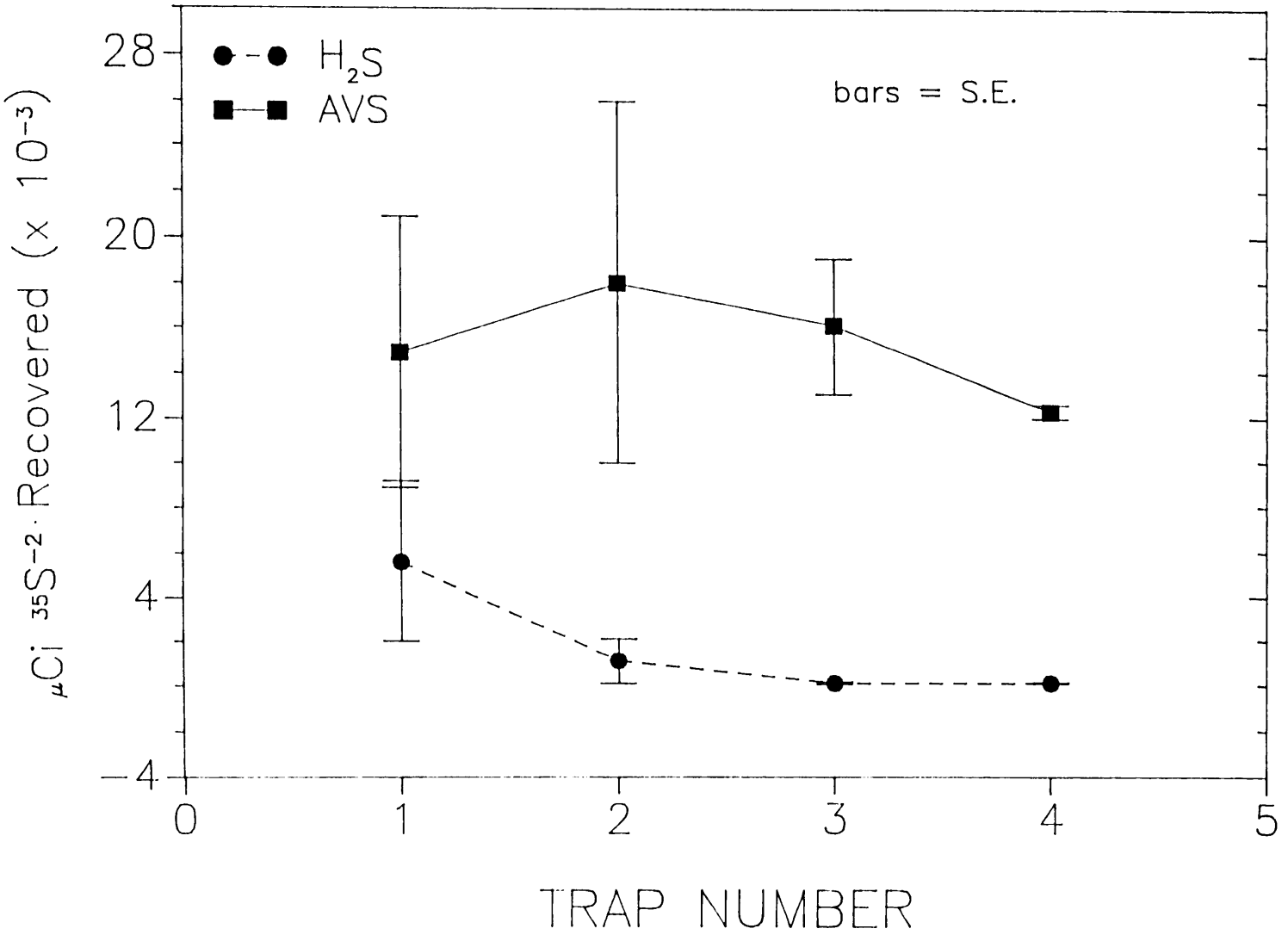
The S^{-2} fractions appeared clearly separated by the water/acid distillation procedure (see Fig. 1.). Jorgensen (8) reported it was previously suggested (Hartman) that sulfides were too unstable to be separated in this way. However, Kaplan et al. (9), Smith (14), and Jorgensen (8) used this type of method to make such distinctions.

Table 1. Summary of ^{35}S cycling in intact black layer micro-cores as influenced by Na_2MoO_4 and NaN_3. One half ml carrier-free $Na^{35}SO_4$ (0.6 uCi ml^{-1}) plus 10% NaN_3 or 10% Na_2MoO_4, was injected into 10 cc intact sediment cores collected directly from a black layer in a 'Penncross' creeping bentgrass golf green. After 48 hrs volatile $H_2{}^{35}S$ from each soil core was distilled for 30 minutes into 2% $CdCl_2$, then counted as $Cd^{35}S$. Next, $AV^{35}S$ was acidified with HCl then distilled for 30 minutes into $CdCl_2$ and again counted. Cores were injected in triplicate.

Sulfide Fraction	Treatment	uCi 35-S Recovered
Free H_2S	10^{-3} M $^{35}SO_4{}^{-2}$	4.40×10^{-3} b
	10^{-3} M $^{35}SO_4{}^{-2}$ in 10% NaN_3	0.10×10^{-3} c
	10^{-3} M $^{35}SO_4{}^{-2}$ in 10% MoO_4	0.10×10^{-3} c
AVS	10^{-3} M $^{35}SO_4{}^{-2}$	94.70×10^{-3} a
	10^{-3} M $^{35}SO_4{}^{-2}$ in 10% NaN_3	0.10×10^{-3} c
	10^{-3} M $^{35}SO_4{}^{-2}$ in 10% MoO_4	0.20×10^{-3} c

Values followed by similar letters were not different by DMRT P=0.05.

Figure 1. Separation of $H_2{}^{35}S$ and $AV^{35}S$ fractions in black layer soil using a S^{-2} trapping train. Radioactive $^{35}SO_4{}^{-2}$ was injected directly into 10 cc black layer micro-cores harvested from a 'Penncross' creeping bentgrass golf green. Accumulated $H_2{}^{35}S$ was distilled from the sample in water. Acidification with HCl volatilized $AV^{35}S$ which was subsequently distilled. The S^{-2} trapping train consisted of a reactor vessel which contained the sample and 4 S^{-2} traps hooked together in sequence and to the reactor via glass line.


28
20
12
4
−4
0
1
2
3
4
5
H_2S
AVS
bars = S.E.
$\mu Ci\ ^{35}S^{-2}$ Recovered (x 10^{-3})
TRAP NUMBER

When Na_2MoO_4 was concurrently injected with $^{35}SO_4^{-2}$, cycling was drastically diminished although some did occurs (see Table 1.). Free $H_2{}^{35}S$ and $AV^{35}S$ were collected in approximately equal concentrations which represented only about 0.03% of the added label each. The effect of the MoO_4^{-2} was to act as a competitive inhibitor of sulfate-reducing bacteria (14). Molybdate was reported to be stereochemically similar to SO_4^{-2}, inhibiting ATP-sulfurylase, the first enzyme in the sulfate-reducing pathway (14). As the inhibition is competitive in nature (14), this means reaction velocity (V_{max}) in the presence or absence of MoO_4^{-2} is similar at a high SO_4^{-2} concentration, but the saturation constant (K_m) is much less in the absence of MoO_4^{-2} (12). Thus, the ability of MoO_4^{-2} to effectively inhibit the activities of sulfate-reducing bacteria would be dependent on the SO_4^{-2} concentration as well as on the concentration of MoO_4^{-2} (14). Smith (14) reported 100% inhibition of SO_4^{-2} reduction in profundal lake sediments with addition of Na_2MoO_4 at only 0.2 mM. Perhaps in our experiment the SO_4^{-2} concentration in the spiked core was great enough (i.e., > 500 nMols added per core) to facilitate some limited reduction in the presence of MoO_4^{-2}. It may have also been that some reduction occurred prior to the MoO_4^{-2} effect, or that the label reached some bacteria while the inhibitor did not.

When NaN_3 was concurrently injected, SO_4^{-2} reduction was again drastically curtailed (see Table 1.). The effect of the NaN_3 was to act as a biocide killing sulfate-reducing bacteria, along with other organisms. Azide combines with the oxidized heme iron of cytochrome a and a_3 preventing their reduction during electron

transport (15). As some cycling of ^{35}S did occur it was probable that SO_4^{-2} reduction occurred prior to the effect of the poison. Another possibility was again that the poison did not physically reach some sulfate-reducers while some $^{35}SO_4^{-2}$ did. These questions were, however, not addressed in the research.

These results of this study prove that SO_4^{-2} reduction was an active, bacterial process in the experimental black layer.

Time Course Studies with a Reactor Vessel. Labeled H_2S was detected with no apparent lag time. The $H_2{}^{35}S$ accrued in a near linear fashion for 24 hours (i.e., $R^2 = 0.99$ $Y' = 7.5 \times 10^{-5} + 6.6 \times 10^{-4}$ (x)). This represented 4.9% of the added label. Acid volatile $^{35}S^{-2}$ recovered after 24 hours represented 8.8% of the added label. The rate of SO_4^{-2} reduction was calculated to be 1.5 nM S cm^{-3} soil day^{-1}. This translated into a mean residence time (12) for the SO_4^{-2} pool (i.e., 4,160 nMols) of 137 days. Residence half life of the labeled SO_4^{-2} was calculated to be 4 days. The calculated rates of SO_4^{-2} reduction for this research were similar to those reported independently by Berner, and Kaplan in the upper 3 m of the Santa Barbra basin (in California), by Sorokin in the surface sediments of the Black Sea, and by Hartman and Nielsen in the surface sediments of the Bay of Keil (8). Our experimentally calculated rates were less than rates reported for the sediments of a eutrophic lake (14) or for the sediment of a model reactor system (8).

When NO_3^- was added concurrently with the label at a level of 48 kg N ha^{-1} S^{-2} production was curtailed. Free $H_2{}^{35}S$ again accrued nearly linearly (i.e., $R^2 = 0.80$ $Y' = 1.7 \times 10^{-4} + 1.8 \times 10^{-5}$ (x) for 24 hours. This however represented only 0.2% of the

added label. Acid volatile $^{35}S^{-2}$ recovered after 24 hours represented only 0.9% of the added $^{35}SO_4^{-2}$. The rate of reduction was calculated to be 0.2 nM S cm^{-3} soil day^{-1}, considerably less than where no NO_3^- was added. This translated into a mean residence time for the SO_4^{-2} pool (i.e., 7,940 nMols) of 1,726 days. Residence half life of the labeled SO_4^{-2} was calculated to be 54 days.

The effect of the added NO_3^- was to act as an alternate electron acceptor, which prevented SO_4^{-2} reduction from occurring by also elevating redox (3, 14). Reduction of NO_3^- was reported to proceed prior to reduction of SO_4^{-2} on thermodynamic grounds. The free energy liberated from reduction of NO_3^- to N_2 was -53 kcal mol^{-1} H_2 while that from reduction of SO_4^{-2} to H_2S was only -9.1 kcal mol^{-1} H_2 (14). Thus, the reduction of NO_3^- is much more spontaneous and would yield more usable energy. However, the effect of the added NO_3^- would be expected to be fairly short lived. Once the NO_3^- pool is depleted through NO_3^- reduction to N_2, SO_4^{-2} reduction should proceed unimpaired. Results of Chapter IV suggest that addition of NO_3^- at 48 kg N ha^{-1} to similar soil under flooded conditions prevented depression of redox for 9 days, after which redox potential declined and S^{-2} production ensued. Thus, frequent NO_3^- addition might be necessary to combat black layer S^{-2} production <u>in situ</u>.

When soils from the study were extracted with water 72% of the added label was recovered where NO_3^- was not added, and 95% of the label was recovered where NO_3^- was applied. For the benzene extract 0.8% of the added label was recovered from both soils. For the NaOBr digest extract the values were 3.3% and 2.9%

respectively. These results indicated that a large percentage of the label remained in the water phase probably as $^{35}SO_4^{-2}$. Additionally, smaller percentages mineralized into elemental ^{35}S and immobilized probably as biomass. Label recovery totals were 89.8% where no NO_3^- was added and 99.8% where NO_3^- was added. Remainder of the label was unaccounted for but a distinct possibility was that where active SO_4^{-2} reduction took place a fraction of the label may have mineralized into-acid insoluble pyrite (FeS_2) or other acid-insoluble oxidation sates. This was not demonstrated in the current research but Howarth (6) showed that pyrite is a major end product in the surface peat of a Cape Cod salt marsh. He further reported that pyrite can form in a day or less without iron mono-sulfides as intermediates. Thus, if the pyrite fraction of the ^{35}S was ignored the rate of SO_4^{-2} reduction would be grossly underestimated. This would in turn magnify the observed differences in rates of reduction where NO_3^- was and was not added.

Reduction of Elemental S. Labeled $H_2{}^{35}S$ was detected with no apparent lag time. The $H_2{}^{35}S$ accrued in a nearly linear fashion for the 24 hour period (i.e., $R^2 = 0.95$ $Y' = 0.29 + 0.02(x)$). This represented 6.4% of the added label. The $AV^{35}S$ recovered after 24 hours represented 25.2% of the added ^{35}S. The rate of elemental S reduction was calculated to be 6.5 nM S cm^{-3} soil day^{-1}. This was very similar to the rates of ^{35}S reduction reported by Smith (14). This translated into a mean residence time for the S pool (i.e., 6,250 nMols) of 54 days. Residence half life of the added label was estimated to be 2 days.

In the supportive experiment it was found that slightly more

than 4x more total S^{-2} accrued where soils were treated with flowable 52% S compared to where soils were treated with SO_4^{-2} as a source of S (see Table 2.). This would agree with projected results considering the stoichiometry of SO_4^{-2} vs S reduction:

$$SO_4^{-2} + 8e^- + 8\ H^+ \ <-----> \ S^{-2} \quad \log K = 20.7$$

$$S + 2e^- + 2\ H^+ \ <-----> \ S^{-2} \quad \log K = 4.9$$

In other words for a given amount of reducing equivalents about 4 times more S^{-2} could theoretically be produced from the reduction of elemental S compared to the reduction of SO_4^{-2}. Thus, from measurement of total S^{-2}, and from observation of reduction of ^{35}S, it appeared that direct reduction of elemental S was occurring. Definitive proof that direct reduction was happening was not collected. To absolutely prove this it would need to be demonstrated that oxidation of ^{35}S to $^{35}SO_4^{-2}$ prior to reduction did not occur. Additionally, it is difficult to accurately access S reduction due to its insolubility in the aqueous phase (14). However, addition of the reducing agent cysteine to the system at the onset of the study should have helped to prevent any ^{35}S oxidation. Also, the fact that nearly 4 times more S^{-2} was produced where S was added (as opposed to SO_4^{-2}) in the supportive study, together with the results of the ^{35}S reduction study was strong evidence that direct S reduction occurred.

It was also very interesting that the observed rate of ^{35}S reduction was some 4 times greater that the measured rate of $^{35}SO_4^{-2}$ reduction in our study with turfgrass soil. Smith (14) reported that the rate of elemental S reduction was less that 40% of the observed rate of SO_4^{-2} reduction at the <u>in situ</u> SO_4^{-2} concentration in profundal surface sediments, and thus, S

Table 2. Influence of added S and NO_3^- on S^{-2} accumulation and redox potential in flooded sand. Sulfur was added as either 52% flowable S or as SO_4^{-2}-S at 48.8 kg S ha^{-1}. Nitrogen was added as NO_3^- at 48.8 kg N ha^{-1}.

Treatments	Sulfides [z]		Redox Parameters		
	H_2S	AVS	pH [y]	pE [x]	pH+pE
Flowable S					
no NO_3^-	8.0 a*	53.2 a	7.5 b	-1.2 b	6.3 b
Flowable S					
+ NO_3^-	2.8 b	1.6 b	7.2 c	2.7 a	9.9 a
Sulfate S					
no NO_3^-	5.2 ab	7.8 b	7.8 a	-2.1 b	6.2 b
$LSD_{.05}$ =	4.4	16.3	0.2	0.6	0.6

z mg S^{-2} kg^{-1} soil solution.

y pH measured with Ag/AgCl combination electrode.

x pE = Eh (millivolts)/59.2.

* Means followed by similar letters were not differenct by LSD @ P = 0.05.

reduction by sulfate-reducers may or may not be of significant magnitude to influence total reduction rates. However, Smith also reported that the two activities may actually involve totally different populations of organisms, since in addition to Desulfuromonas, photosynthetic bacteria and cyanobacteria were shown to reduce elemental S in the dark. Thus, different populations of S or SO_4^{-2} reducers may exist in turfgrass soils. Alternatively, it may be that the total populations of sulfate-reducers in our experimental soil may have differed greatly from point to point, reflected in our sampling.

CONCLUSIONS

This research has demonstrated that active S cycling of both SO_4^{-2} and elemental S occurs in black layer soils. The cycling was shown to be bacterial in nature. Further, it was shown that addition of NO_3^- at traditional levels had an antagonistic influence on rates of SO_4^{-2} (or S) reduction. The research results have also lended support to the hypothesis that black layer formation involves bacterial SO_4^{-2} or S reduction.

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