CHAPTER IV

INFLUENCE OF SULFUR, AMMONIUM AND NITRATE ON IN VITRO SULFIDE ACCUMULATION AND REDOX POTENTIAL

Abstract. Previous research has suggested that black layer in turfgrass soils was an accumulation of S^{-2}, and that S addition positively influenced S^{-2} formation. Fertilizer NO_3^- was also previously shown to prevent S^{-2} accumulation presumably by keeping redox potential elevated. Whether other N sources would prevent S^{-2} accumulation was unknown. Thus, the influence of S, NH_4^+, and NO_3^- on S^{-2} accumulation and redox potential in flooded soil from a 'Penncross' creeping bentgrass green was studied in the laboratory. Addition of flowable S to flooded soil at levels of 48 kg ha^{-1} caused an increase in free H_2S and in acid volatile S^{-2} compared to other treatments. Concurrent addition of N as NH_4^+ at 48 kg ha^{-1} retarded acid volatile S^{-2} accumulation but did not prevent formation of free H_2S. Nitrate was found to be considerably more effective at retarding S^{-2} production, as no H_2S was detected where NO_3^- was added, and AVS production was delayed by 6 days compared to other treatments. Nitrate was also shown to prevent redox potential depression where NH_4^+ did not. It was concluded that S contributed to and N retarded S^{-2} formation. It was also concluded that a critical pE + pH was necessary for S^{-2} formation, and that sufficient NO_3^- from fertilization may help to prevent S^{-2} production in turfgrass soils by maintaining a pE + pH above the critical point.

Black layer formation in highly maintained turfgrass soils has been related to an accumulation of S^{-2} in the profile (1, 9, 11). A major effort in black layer research was to find acceptable methods of black layer control which could be used by turfgrass practitioners. Core aerification, reconstruction of afflicted areas, installation of additional drainage and fungicide application have been used to combat black layer, all with limited success at best.

Sulfide formation in flooded rice (Oryza sp) paddy soils was shown to be dependent on redox potential (2, 4, 5, 7, 14). Connell and Patrick (2) reported little S^{-2} production in rice paddy soils with a redox potential above -150 mV. Likewise, Engler and Patrick (4) reported that SO_4^{-2} reduction and S^{-2} oxidation in rice paddys occurred in the vicinity of -100 mV. However, Vainsthein (14) reported that a SO_4^{-2} reduction process and sulfidic compounds (pyrite) were detected in Indian Ocean sediments with redox potential near +500 mV. In experiments with an apparatus to modify redox with active H_2, Vainsthein reported active SO_4^{-2} reduction by Desulfovibrio baculatus at +120 mV. He reported that redox status probably affected SO_4^{-2} reducer type more than the actual process. Thus, depending on the type of SO_4^{-2} reducers present, S^{-2} production may take place over a wide range of redox potentials.

The effects of various chemical oxidants to inhibit S^{-2} formation in flooded rice paddy soils were also studied (4, 7). It was found that if redox potential was kept high, S^{-2} production was delayed and suffocation disease of rice was prevented. Results further indicated that oxidants which were soluble, such as NO_3^- and ferriocitrophosphate, had the greatest effect in maintaining high redox potentials, hence in preventing S^{-2} production. Less soluble oxidants such as MnO_2 and $FePO_4$ were not as effective but persisted longer. The objective of this research was to determine the effect of added S, NH_4^+ and NO_3^- on the redox potential and S^{-2} formation tendencies of flooded turfgrass soil.

MATERIALS AND METHODS

Thirty grams of sand (88.3% sand, 5.0% silt and 6.7% clay) from a 'Penncross' creeping bentgrass (Agrostis palustris Huds.) golf green located at the Hancock Turfgrass Research Center in East Lansing was added to 30 ml glass anaerobe tubes (Bellco Glass). Soil was collected to a depth of 15 cm, then air dried, homogenized, and sieved to 500 um. Root tissue retained on the sieve was ground to 20 mesh with a Wiley mill and added back to the soil and mixed. Soil material not passing 500 um was discarded. The composite soil had a C:N of 11.4:1. Most probable number estimates (12) of sulfate-reducing bacteria in the composite soil incubated in a flooded state for 2 weeks were 1.7×10^6 SRBs per gram of soil. Where S was added to this soil at 48 kg S ha^{-1} the SRB numbers increased to 2.2×10^6.

Tubes containing soil were then flooded with tap water so that the soil was entirely under water, and flowable S (52%) was added at a level of 48.8 kg S ha^{-1}. Sulfate content of the tap water was approximately 30 ug ml^{-1}. Flooded tubes to which no S was added were left as controls. Tubes were not shaken or inverted to mix the soil and S. Mixing was averted so that S would come to rest on the flooded soil surface simulating field application. One third of the S treated tubes also received NH_4^+-N from NH_4Cl in solution at a level of 48.8 kg N ha^{-1} while another third received NO_3^--N from $Ca(NO_3)_2$ also at 48.8 kg ha^{-1}. There were three replications of each treatment (i.e., no S, S only, S + NH_4^+ or S + NO_3^-). Sufficient tubes of each treatment were prepared to enable daily analysis of each treatment for 18 days including day 0. After treatment with S and N, additional tap water was added to each tube so that no headspace existed. Each tube was stoppered with butyl rubber and crimped with aluminum seals to halt O_2 diffusion and initiate anaerobiosis. Microbial respiration was assumed to initiate the anaerobiosis.

Three tubes from each treatment were analyzed daily for accumulation of free H_2S, AVS, and redox potential as pE + pH (6). Measurement of free H_2S using the method of Cord-Ruswich (3) was performed first, by withdrawing 2-3 mls of soil solution from closed tubes with a syringe. To do this a de-aired 5 ml syringe was inserted through the stopper so that the needle opening was under water. Next another syringe containing N_2 was inserted and the N_2 was injected to displace tube water, which entered the first syringe through a 0.2 um filter. In this way the filtered soil solution was sampled without interference from atmospheric O_2. Stoppers were then removed under a stream of O_2-free N_2 and pH and Eh were determined. Soil solution reaction was measured with a Ag/AgCl combination electrode and Eh of surface soil measured with a Pt electrode with calomel reference. A salt bridge was necessary to measure Eh. An Orion 811 microprocessor served both electrodes. Lastly, AVS was measured by replacing withdrawn water with de-aired distilled water, re-stoppering then subsequently injecting 2 ml 37% HCl. The acid solubilized AVS which was then measured in the same way as H_2S.

RESULTS AND DISCUSSION

Soil treated with S produced more acid volatile S^{-2} (AVS) after 18 days than a control soil (see Fig. 1). A final S^{-2} concentration of 885 mg kg^{-1} for soil treated with S, compared to a concentration of 165 for the control, represented a S^{-2} increase of 536%. At the end of the experiment the AVS in tubes where S was added was visible as intense black banding beginning at the soil/water interface. Banding extended from the interface to 4-5 mm below the soil surface. No banding appeared below 5 mm. Soil past that depth had a gleyed appearance characteristic of reduced soils. Likewise, in control units no banding occurred, but the gleyed appearance was evident. Upon closer examination of the soil in control tubes, and in soil in S treated tubes below the banding, distinct semi-micro areas of intense blackening were also observed. These were probably formed in response to proximity to organic debris.

Concurrent addition of N as either NH_4^+ or NO_3^- with S retarded the production of AVS. This response was expected with addition of NO_3^- as it is a well established alternate electron acceptor (2, 12). The standard free energy of reduction of NO_3^- was reported to be -53.6 kcal mol^{-1} H_2 consumed, while that of SO_4^{-2} or S was -9.1 and -6.7 respectively (12). Thus more

Figure 1. Influence of S, NH_4^+, and NO_3^- on accumulation of AVS in flooded soil from a 'Penncross' creeping bentgrass golf green. Sulfide production was enhanced by addition of S while concurrent addition of N as either NH_4^+ or NO_3^- retarded it. Both S and N were applied at 48 kg ha^{-1}. Means were the averages of 3 observations per day over 18 days.

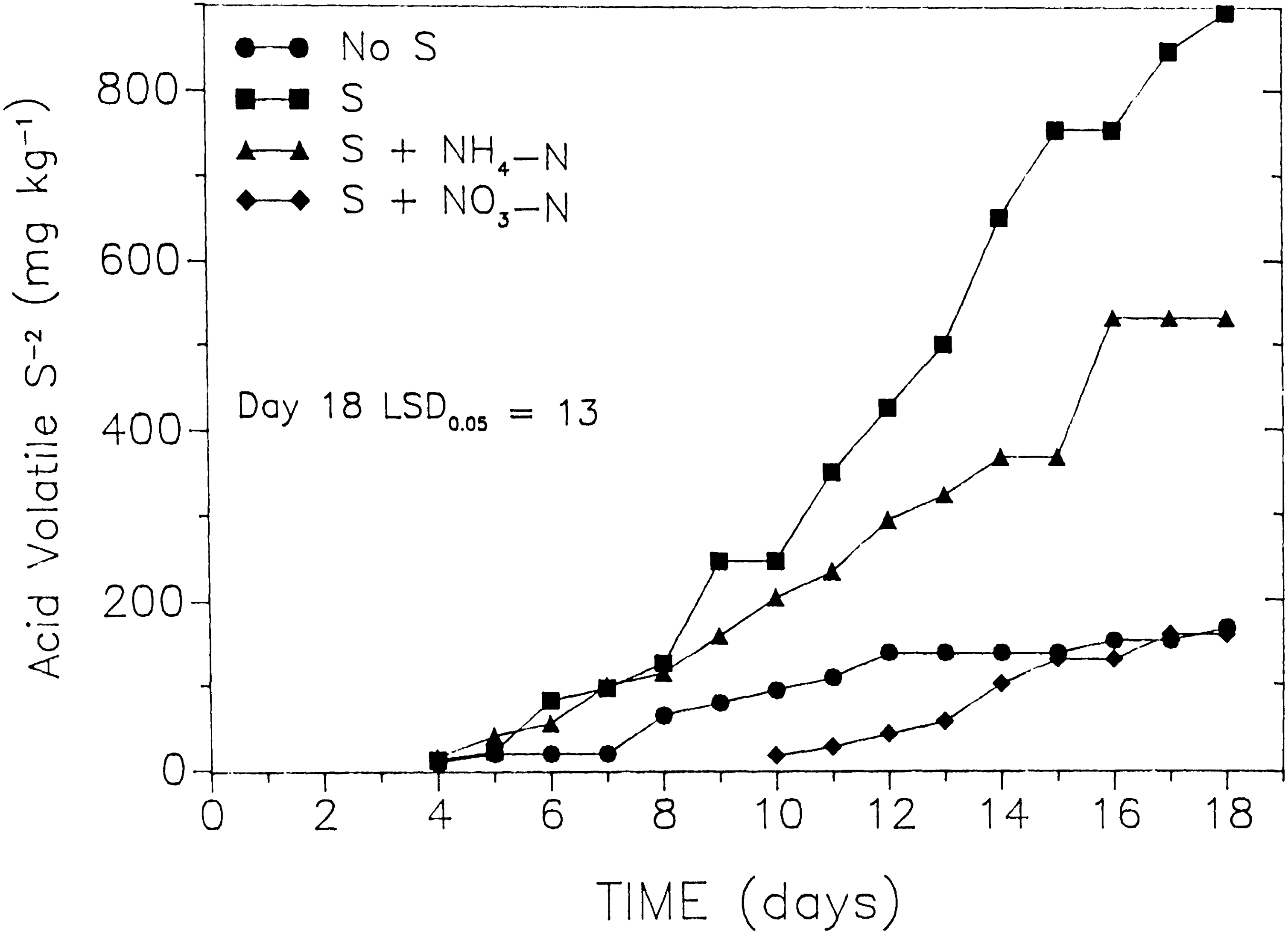

No S
S
S + NH_4–N
S + NO_3–N
Day 18 $LSD_{0.05}$ = 13
Acid Volatile S^{-2} (mg kg^{-1})
0
200
400
600
800
0
2
4
6
8
10
12
14
16
18
TIME (days)

Table 1. Linear regression statements applied to acid volatile S^{-2} accumulation as influenced by S, NH_4^+, or NO_3^-. Regressions were calculated by the method of least squares.

	Regression Parameters		
Treatment	R^2	Slope	Intercept
No S	0.93**	12.41	-44.18
S	0.97**	69.25	-367.42
S + NH_4-N	0.97**	40.27	-193.61
S + NO_3-N	0.96**	20.67	-208.78

*,** Denotes significance at P = 0.05 and P = 0.01 respectively.

Strength of association.

Ordinate intercept.

metabolic energy for biomass would be derived from NO_3^- metabolism compared to metabolism on SO_4^{-2} or S. In other words SO_4^{-2} or S reduction (hence AVS formation) should have been "by-passed" in the presence of NO_3^- until the available NO_3^- pool was depleted. After that SO_4^{-2} or S reduction should proceed unimpaired. Indeed, AVS formation in the tubes treated with NO_3^- was delayed by 6 days compared to tubes treated with only S. After that time AVS formation proceeded at a relatively quick pace. Likewise, Engler and Patrick (4) and Connell and Patrick (2) reported that addition of NO_3^- to flooded rice paddy soil delayed SO_4^{-2} reduction but did not decrease the rate of S^{-2} formation once it commenced.

The response to NH_4^+ was more intriguing. It was initially assumed that the highly reduced NH_4^+ would help to generate more AVS than S alone by providing the preferred N source for sulfate-reducing bacteria (8). Probably what happened was a portion of the NH_4^+ oxidized to NO_3^- in the first few days of the experiment effectively increasing the NO_3^- concentration (10). Although NO_3^- content was not measured in this study this conclusion seemed reasonable since highly aerobic tap water was used to flood the tubes, and nitrifying bacteria should be present in the soil.

Regression analysis of the AVS accumulation curves (see Table 1.) indicated the production of AVS to be highly linear (i.e., $R^2 > 0.9$ for all curves) with day 4 as the origin. The slope of the S treatment was the steepest followed by the NH_4^+ curve, the NO_3^- curve and finally the control.

Free H_2S formation was stimulated by addition of S or S + NH_4^+ (see Fig. 2). It was not detected where S was not added, or where NO_3^- was added concurrently. Free H_2S was first detected on day 11, some 7 days after the formation of AVS was noticed. Once production began the rates of accumulation were similar for those treatments. Linear regression analysis indicated a slope of 15(x) for both the S and S + NH_4^+ treatments, and a slope of 0 for remaining treatments. This result implied that potential for turf plant loss due to stress imposed from the presence of H_2S was heightened if S was in abundance or if ammonical fertilizers were applied with S. Results also implied that S and not organic matter was limiting for production of free H_2S. This would concur with results generated in Chapter III where it was determined that a critical level of S was still necessary to generate free H_2S when organic matter was not limiting.

Depression of redox potential as pE + pH was nearly linear for the first 3 days of the study (see Fig. 3). The pE + pH declined from near 16 to near 7 in 18 days regardless of treatment. The soil treated with only S appeared have a slightly lower pE + pH initially (i.e., day 3) but the effect was short lived as no differences between the S, no S, or S + NH_4^+ treatments were evident at day 5. A possible explanation for this initial difference may have been due to the oxidation of S to SO_4^{-2} consuming O_2 in the process. Where NO_3^- was added with S, either concurrently or from NH_4^+ oxidation, sufficient O_2 was probably present to offset the effect. Where no S was added no scavenging of the O_2 occurred. No other differences were detected between these treatments during the rest of the course of the study.

Figure 2. Influence of S, NH_4^+, and NO_3^- of accumulation of H_2S in flooded soil from a 'Penncross' creeping bentgrass golf green. Free H_2S production was enhanced by addition of S or S + NH_4^+ while addition of NO_3^- prevented it. Also, no free H_2S was detected where S was not added. Both S and N were applied at levels of 48 kg ha^{-1}. Means were the averages of 3 observations per day for 18 days.

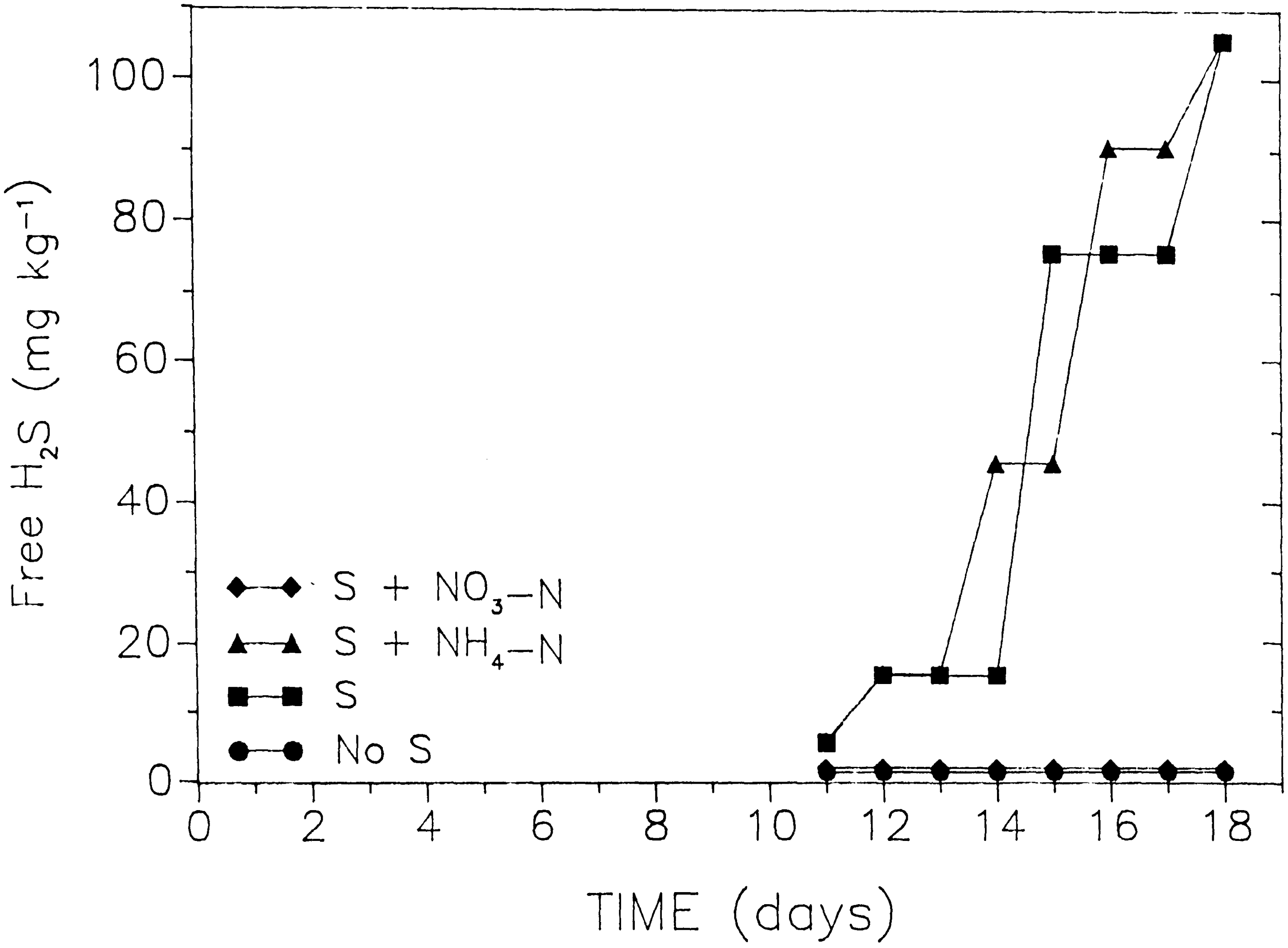

Free H_2S (mg kg^{-1})
0
20
40
60
80
100
0
2
4
6
8
10
12
14
16
18
TIME (days)
S + NO_3–N
S + NH_4–N
S
No S

Figure 3. Influence of S, NH_4^+, and NO_3^- on redox potential as pE + pH in flooded soil from a 'Penncross' creeping bentgrass golf green. Redox potential was calculated with a Pt redox electrode and Ag/AgCl pH electrode. Only NO_3^- prevented the depression of redox which appeared to control S^{-2} production. Means were the averages of 3 observations per day for 18 days.

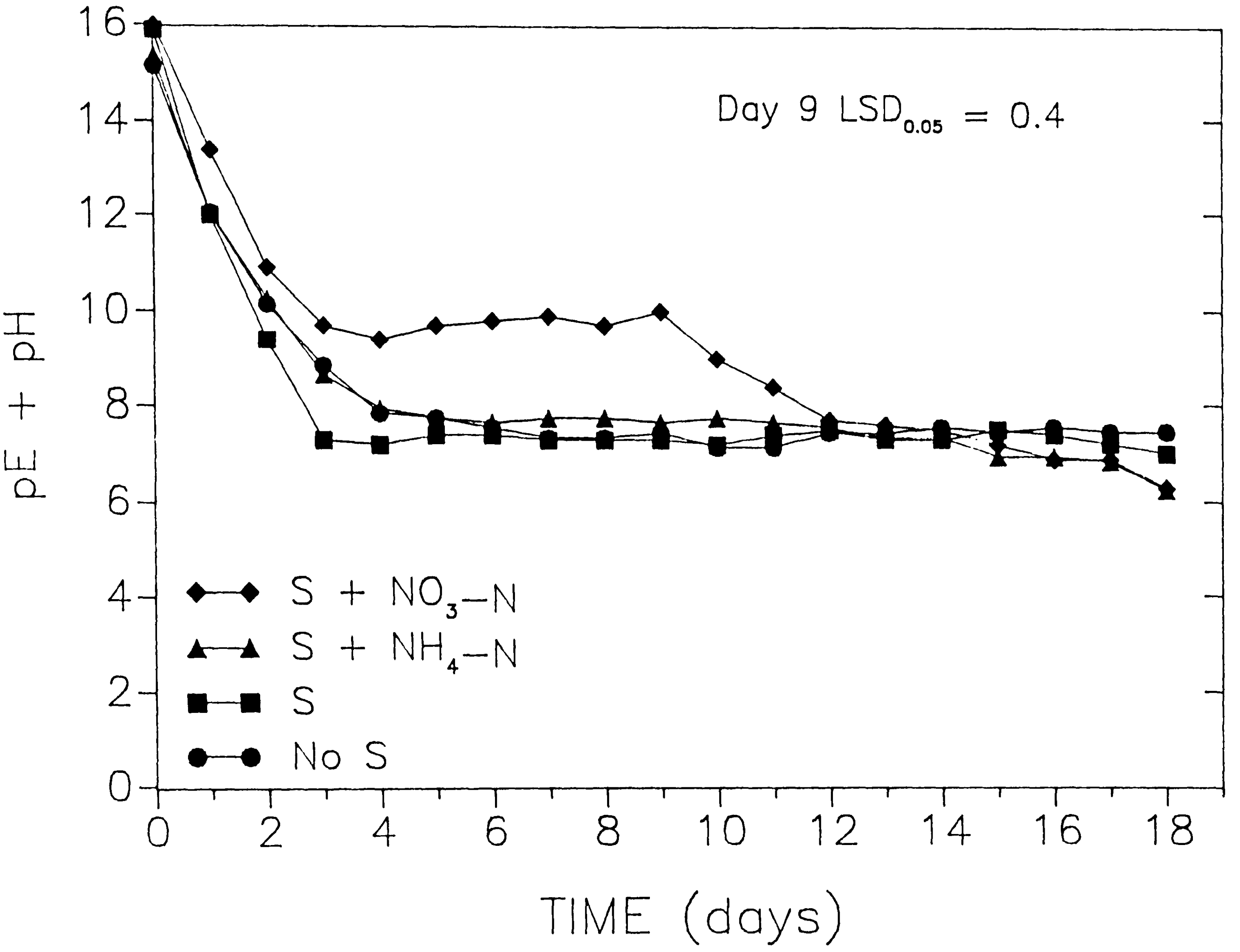
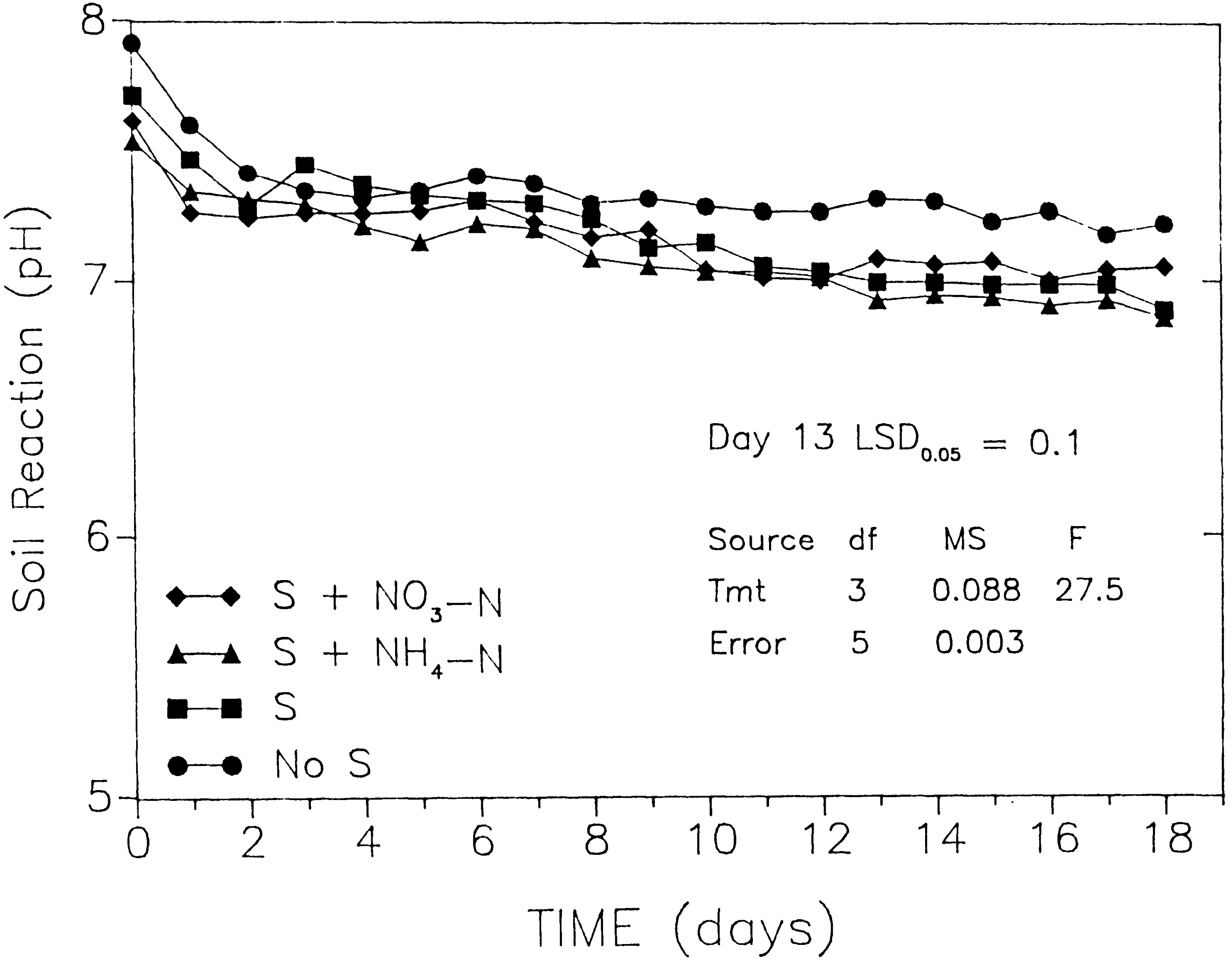

16
14
12
10
8
6
4
2
0
pE + pH
0
2
4
6
8
10
12
14
16
18
TIME (days)
Day 9 $LSD_{0.05}$ = 0.4
◆—◆ S + NO_3–N
▲—▲ S + NH_4–N
■—■ S
●—● No S

Figure 4. Influence of S, NH_4^+, and NO_3^- on pH of flooded soil from a 'Penncross' creeping bentgrass golf green. Soil reaction was measured with a Ag/AgCl combination electrode. Means were the averages of 3 observations per day for 18 days.



Day 13 $LSD_{0.05} = 0.1$

Source	df	MS	F
Tmt	3	0.088	27.5
Error	5	0.003	

Only the NO_3^- treatment significantly retarded pE + pH depression. The effect of the NO_3^- was evident early on and lasted several days. At day 8 however, the effect of added NO_3^- waned, and pE + pH dropped to 7.5 by day 12. By day 18 no differences between any treatments were apparent. The decrease in pE + pH to near 9 at day 10 for the S + NO_3^- treatment coincided with the appearance of AVS for that treatment. Thus it appeared that a pE + pH of near 10 or above was sufficient to prevent sulfide formation. For treatments where pE + pH values were less than that, S^{-2} formation was imminent. Likewise, for the other treatments lowering of pE + pH to near 9 on or around day 4 also coincided with production of S^{-2}. Thus, it appeared that redox was a controlling factor in the production of S^{-2}.

CONCLUSIONS

This research has demonstrated that S intensified S^{-2} production and N retarded it. Nitrate was shown to prevent S^{-2} production by acting as an alternate TEA while also keeping redox above a critical level. The effect of NO_3^- was short lived and S^{-2} production was probably initiated only after the pool of NO_3^- was converted to N_2. Thus, it appeared that NO_3^- was a potential control for S^{-2} based black layer.

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