RESEARCH UPDATE ON BLACK LAYER

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There has been a lot of controversy regarding anaerobic black layer. We at Michigan State University contend that black layer formation involves dissimilatory sulfate reduction which produces gaseous hydrogen sulfide. We believe that physical formation of the layer is due to a reaction between biologically produced hydrogen sulfide and common minerals such as iron, under anaerobic conditions. The reaction produces precipitated metal sulfides, which accumulate in soil pore spaces. These precipitates are presumed non-toxic but may accumulate to the extent that oxygen diffusion and water drainage are impeded. In theory, the chemistry of sulfide allows it to "scavenge" diffusing oxygen making it less available. The turf decline which appears concurrently with black layer is believed to result from the occurence of free hydrogen sulfide and other toxic metabolites, such as acetic acid, which persists under fermentation conditions (i.e., anaerobiosis).

Qualitative research on black layer at M.S.U. supports the sulfate reduction hypothesis. Several experiments were undertaken in an attempt to demonstrate the relationship of sulfur and sulfate reduction to black layer formation, the biological nature of this activity, and potential avenues of black layer control.

Study 1.

This experiment was initiated at a glasshouse facility at Michigan State University in East Lansing, MI. The objective of Study 1 was to test the proposed relationship between sulfur application and black layer formation.

Experimental units consisted of 2-liter plastic buckets fitted with air tight lids and having several small holes (i.e., 1/8") drilled in the bucket bottom. This arrangement diminished air diffusion into the bucket from the top but allowed for free passage of water from the bottom. The buckets were packed with a medium-fine Lake Michigan dune sand topped by 1 inch of fine mortar sand, thus creating a perched water table situation. This arrangement helped to hold water high in the soil profile. The sands were treated with 1 or 5 pounds of sulfur (flowable, 52% S) and 2 ounces of iron (ferrous sulfate) per 1000 square feet, and inoculated with lactate enriched suspensions of mixed cultures of sulfate reducing bacteria. The treated soils were then waterlogged and made anaerobic by attaching the lids and immersing the bucket bottoms in 5 inches of water for 33 days. Check buckets with sands receiving no sulfur were also prepared and waterlogged in an identical manner. At the end of 33 days, each bucket was disassembled and visually examined for evidence of black layer.

Sulfur treated sands readily formed black layer within 33 days but no black layer formed in sands lacking sulfur applications. A more intense black color was observed where more sulfur was added and also in the finer textured mortar sand. Without added sulfur, no black layer formed even though anaerobic conditions prevailed and sulfate reducing bacteria were present. Algae grew on the surface of the sulfur treated sands, but preliminary observations indicated that black layer formation occured prior to extensive algal growth. These results suggested that sulfur was involved in black layer formation and that algae was a secondary invader.

Study 2.

This experiment was initiated at the glasshouse facility previously described. The objective of Study 2 was to demonstrate that the formation of black layer was biological, and that formation could be delayed by addition of alternate electron acceptors. Another objective was to demonstrate the relationship between black layer and sulfate reduction with the use of specific inhibitors of sulfate reduction.

Buckets (as previously described) were packed with sand (as described) and treated with 15 pounds of sulfur (re-precipitated elemental) per 1000 square feet. Packing was adjusted so the sulfur could be placed 3 inches below the sand profile surface. Lactate enrichments of mixed cultures of sulfate reducers were also added. In addition, 200 mls of 0.1 M bleach, potassium nitrate, sodium nitrate, calcium nitrate, or ammonium molybdate were added to each of 3 experimental sulfur treated units (buckets). Three sulfur treated units not amended with bleach, nitrate or molybdate served as active black layer forming checks. The units were then waterlogged and made anaerobic (as described) for 14 days. At the end of 14 days the units were disassembled and visually inspected for black layer formation.

Sulfur amended sands treated with bleach, nitrate or molybdate showed no evidence of black layer after 14 days. Sulfur amended sands receiving no bleach, nitrate or molydate treatment actively formed black layer. The results suggested black layer formation to be a biological process which was manipulated by addition of alternate electron acceptors (i.e., nitrates) and bleach. Nitrate additions were presumed to influence the respiratory pathways of the microbial populations. The bacteria probably derived more energy from the respiratory utilization of nitrates as electron acceptors than was possible with sulfate, hence sulfate reduction was "bypassed." It was also presumed that the bleach acted as a disinfectant effectively killing sulfate reducers, since previous additions of bleach to crude cultures of sulfate reducers in iron free medium halted formation of hydrogen sulfide. It was possible that the nitrate compounds and bleach also acted as oxidizing agents which dissipated the reduced black layer as it formed. This was, however, not determined. The use of compounds which serve as alternate electron acceptors or oxidants may prove to be effective black layer control, but more research is needed.

When molybdate was added, as a specific inhibitor of sulfate reduction, sulfur amended sands did not form black layer. This fact strongly suggested black layer formation to be an active sulfate reduction process and was more evidence linking black layer formation to sulfur.

Study 3.

This experiment was initiated at the glasshouse facility previously described. The objective was to determine whether sulfur additions would produce a black layer in turfed soils taken directly from the field and if so whether nitrates would suppress layer formation.

Plugs of turfed soil from a Penncross creeping bentgrass green were harvested and transplanted to 2-liter plastic buckets which were previously described. Harvested plugs were approximately 10.5" x 4.25". Washed dune sand was used to backfill around the exposed edges of the plugs inside the buckets. Two pounds of sulfur (flowable, 52%) per 1000 square feet were applied to 3 of the plugs while 3 other plugs received 2 pounds of sulfur plus 2 pounds of N from calcium nitrate per 1000 square feet. Three additional plugs were left untreated as checks. The experimental units were then waterlogged for 14 days as previously described. After 14 days, the units were disassembled and visually inspected for evidence of black layer.

Penncross turf plugs treated with 2 pounds of sulfur per 1000 square feet formed black layer within 14 days after waterlogging. When similar plugs received 2 pounds of sulfur plus 2 pounds of N from calcium nitrate per 1000 square feet no black layer was formed. Likewise, no black layer was formed in untreated checks. Thus, the results again suggested a relationship between sulfur application and black layer formation. The results also suggested that nitrate application prior to black layer formation was an effective preventative control method.

Summary

The results of these experiments suggested a strong relationship between the application or occurence of sulfur and black layer formation. Through the use of bleach and molybdate black layer formation was shown to be a biological process involving dissimilatory sulfate reduction. Black layer formation was also shown to be delayed by application of nitrates. However, examination of the relationships of black layer formation regarding other common cultural practices, such as irrigation and fertility, is needed. Also, more research must be conducted regarding the basic mechanism of black layer suppression when nitrates are introduced.