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# Can Denitrification Reduce $\text{NO}_3$ in Shallow Ground Water?

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# agronomy notes

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## CAN DENITRIFICATION REDUCE $\text{NO}_3^-$ IN SHALLOW GROUND WATER?

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### INTRODUCTION

Systematic sampling of springs, tiles, and wells in Kentucky, as part of a recent statewide program to assess agricultural impacts on water quality, showed that  $\text{NO}_3^-$  concentrations in these shallow ground water sources varied tremendously. The  $\text{NO}_3^-$  concentration could be correlated with flow rate; higher when ground water recharge flushed  $\text{NO}_3^-$  from soil in winter and spring, and lower or non detectable in summer and fall when less  $\text{NO}_3^-$  leaching occurred. Depending on the season,  $\text{NO}_3^-$  concentrations ranged from <1 to >10 ppm  $\text{NO}_3^-$ -N in almost half of the sites. For example, the water in one site, a shallow well over a naturally occurring spring in Bourbon county, varied from 0 to 12 ppm  $\text{NO}_3^-$ -N during the year (Figure 1).

There is an alternative explanation for this variability, an explanation that isn't based on ground water recharge events. An interaction between flow rate and biological activity could explain some of the variability of  $\text{NO}_3^-$  concentration in this and similar sites. Since the water percolated through a sediment layer in the Bourbon county well before it could be sampled, it seemed likely that biological denitrification (a

microbial process in which bacteria convert  $\text{NO}_3^-$ -N to  $\text{N}_2$  gas) during low flow periods might account for the low  $\text{NO}_3^-$  concentrations. When water flow was high,  $\text{NO}_3^-$  movement through the sediment layer would be too rapid for complete biological removal. We tested this idea by recreating flow-dependent  $\text{NO}_3^-$  concentrations in a series of laboratory studies.

### METHODS

We collected sediment from the spring-fed well in Bourbon county and used it to fill 7-inch-tall PVC cylinders about half full. During an experiment, a 10 ppm  $\text{NO}_3^-$ -N solution was pumped into the bottom of the cylinders at either a fast, slow, or intermediate rate (10 ppm is the maximum allowable  $\text{NO}_3^-$ -N concentration for drinking water in Kentucky). Outflow at the top of the cylinders was analyzed for  $\text{NO}_3^-$ -N, nitrite  $\text{N}$  ( $\text{NO}_2^-$ -N), and ammonium  $\text{N}$  ( $\text{NH}_4^+$ -N). We also used an inhibitor to stop the last step in denitrification, and measured the intermediates that accumulated. Two cylinders were used for each experiment to show that the results were reproducible. Multiple experiments were conducted, and multiple measurements were

taken within each experiment to demonstrate that the trends were consistent.

## RESULTS AND DISCUSSION

The  $\text{NO}_3^-$  concentrations leaving the cylinders in the laboratory varied as they had in the spring-fed well; they were highest when flow rates were highest, lowest when flow rates were lowest, and of intermediate concentration when flow rates were intermediate (Figure 2). Since the  $\text{NO}_3^-$  concentration in the water supply was constant, something other than flow caused the changes in  $\text{NO}_3^-$  concentration in the sediment outflow. We assumed that by creating laboratory conditions which reproduced  $\text{NO}_3^-$ -N variability in the spring-fed well, we could also reproduce mechanisms causing that variability.

When oxygen becomes deficient in waterlogged soils (because microbes can consume oxygen faster than it is supplied by flowing water) a biological process such as  $\text{NO}_3^-$  reduction can occur. The rate of  $\text{NO}_3^-$  reduction depends on the size and activity of the microbial population, and how long they have access to  $\text{NO}_3^-$ . If the rate of  $\text{NO}_3^-$  flowing into sediment was less than the rate at which it was reduced, then  $\text{NO}_3^-$  concentrations would decline. That's exactly what happened when flow rates decreased (Figure 2). If  $\text{NO}_3^-$  flow through the sediment exceeded the  $\text{NO}_3^-$  reduction rate, then  $\text{NO}_3^-$  concentration should rise; this also happened (Figure 2). As soon as the  $\text{NO}_3^-$  flow rose to an intermediate rate, the  $\text{NO}_3^-$  concentration in the sediment outflow increased. It didn't increase to its original level, which meant that some of the  $\text{NO}_3^-$  was still being reduced.

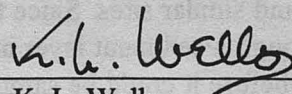
The  $\text{NO}_3^-$  could disappear by being converted to  $\text{NH}_4^+$ . If  $\text{NO}_3^-$  were reduced to  $\text{NH}_4^+$ , the  $\text{NH}_4^+$ -N concentration should have increased as flow rate decreased. However, the  $\text{NH}_4^+$ -N concentration in sediment didn't change much when flow rate changed (Figure 3) even though

$\text{NO}_3^-$  concentrations fluctuated.

The  $\text{NO}_3^-$  could disappear by being converted to  $\text{N}_2$  (denitrification). We can't measure nitrogen gas ( $\text{N}_2$ ), the final product of denitrification, as easily as  $\text{NH}_4^+$ . However, if we found nitrous oxide ( $\text{N}_2\text{O}$ ), an intermediate of denitrification which can be detected easily with a gas chromatograph, it would be evidence that denitrification removed  $\text{NO}_3^-$ . Acetylene ( $\text{C}_2\text{H}_2$ ) inhibits  $\text{N}_2\text{O}$  reduction to  $\text{N}_2$  and causes  $\text{N}_2\text{O}$  to accumulate. So, if denitrification was the reason  $\text{NO}_3^-$  disappeared,  $\text{N}_2\text{O}$  would appear in the headspace of the cylinders once  $\text{C}_2\text{H}_2$  was added, and flow was reduced. Just as we expected,  $\text{N}_2\text{O}$  appeared as soon as we added  $\text{C}_2\text{H}_2$ . Denitrification was clearly involved in  $\text{NO}_3^-$  reduction in this sediment, and based on the initial and final  $\text{NO}_3^-$ -N concentrations we observed during these experiments, it removed between 60 and 68% of the added  $\text{NO}_3^-$ .

## CONCLUSION

Assessing agriculture's contribution to  $\text{NO}_3^-$  contamination of ground water has been difficult because of varying  $\text{NO}_3^-$  concentrations. In addition to fluctuating  $\text{NO}_3^-$  concentrations due to ground water recharge, some of the variability of  $\text{NO}_3^-$  concentrations in watersheds could be due to biological denitrification. Our results indicate that when conditions are right for denitrification (for example, low flow, long residence time, poor water recharge and oxygenation, and abundant carbon),  $\text{NO}_3^-$  in shallow ground water can be reduced if it percolates through saturated layers of sediment.



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Figure 1. Nitrate N concentrations measured in a spring-fed Bourbon County well for 1 year.

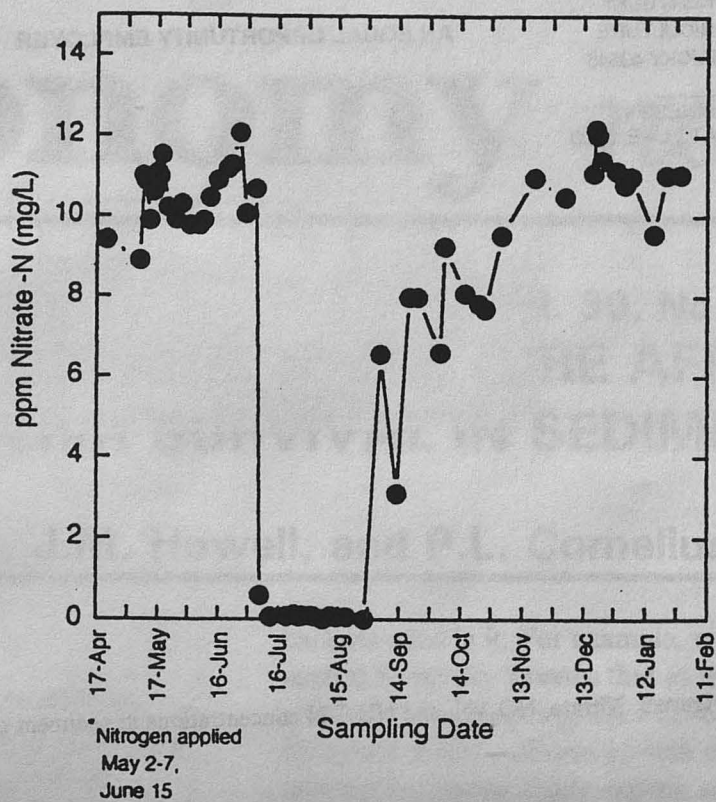
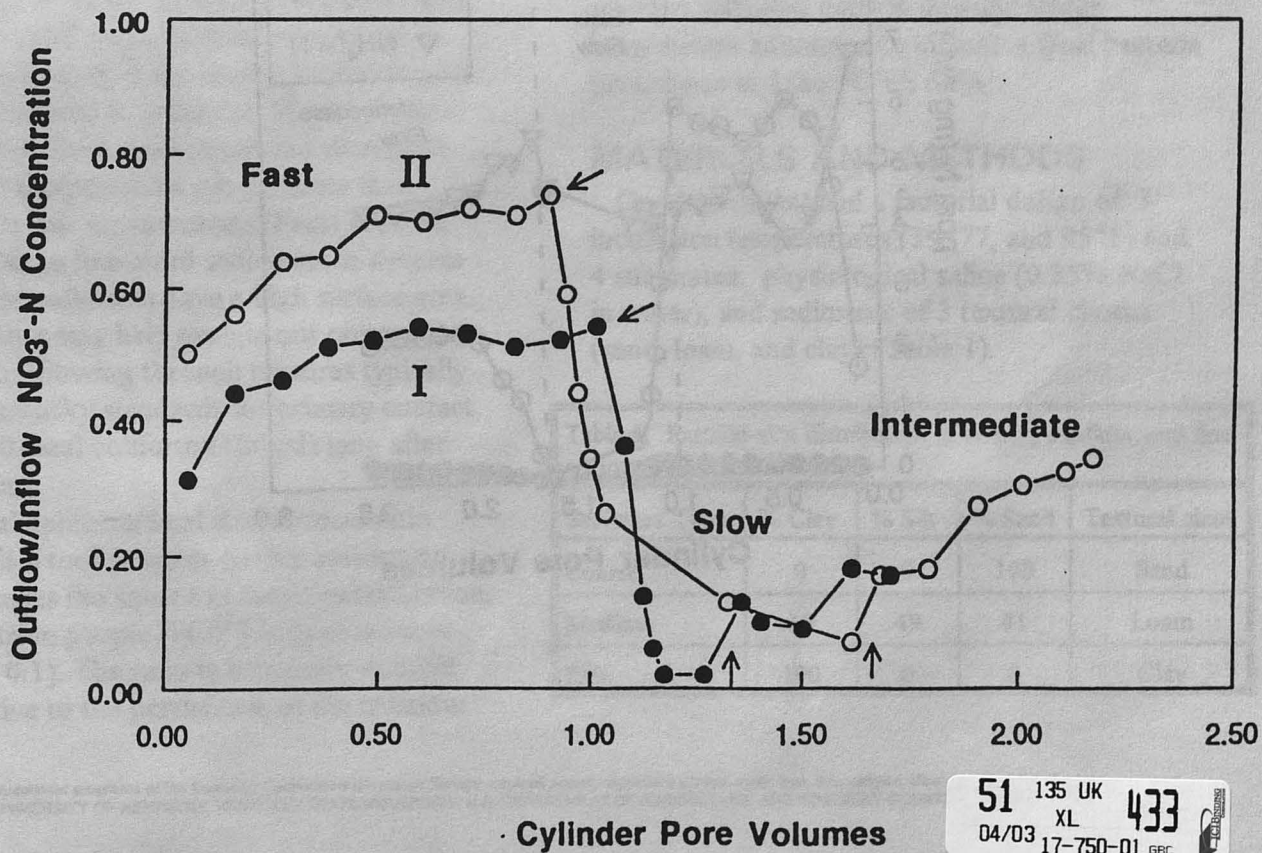


Figure 2. Ratio of  $\text{NO}_3^-$ -N concentrations in outflow and inflow from a sediment with variable flow rates. Arrows indicate when flow rates were changed (fast  $\leftrightarrow$  slow  $\leftrightarrow$  intermediate) in two replicates (I & II).



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Figure 3. Nitrate,  $\text{NO}_2^-$ -N, and  $\text{NH}_4^+$ -N concentrations in sediment subjected to variable flow.

