

Wijler J & Delwiche C C. Investigations on the denitrifying process in soil.
Plant Soil 5:155-69, 1954.
[Department of Plant Biochemistry, University of California, Berkeley, CA]

Using isotopically labeled nitrate ion, it was possible to distinguish processes of denitrification from other simultaneous nitrogen-cycle reactions in soil. The production of nitrous oxide and nitric oxide in the reaction and their later reabsorption were related to oxygen tension, pH, and other variables. [The *SCI*® indicates that this paper has been cited in over 130 publications since 1955.]

C.C. Delwiche
Department of Land, Air and
Water Resources
University of California
Davis, CA 95616

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This work resulted from the fortunate convergence of a number of factors: by the 1950s the overall picture of denitrification in pure cultures, including the formation and reassimilation of nitrous oxide, had been well established,¹ but it had not been possible to isolate denitrification from other nitrogen-cycle reactions in mixed dynamic systems such as soil. Only the net result of simultaneous and competing reactions had been observed. Enrichments of the rare stable isotope of nitrogen (¹⁵N) had become available, making possible the examination of features of the nitrogen cycle that previously had been difficult to isolate.

Stable tracers imply the need for a mass spectrometer, and fortunately, through the efforts of the late W.M. Stanley, director of the Biochemistry and Virus Laboratory at the University of California, Berkeley, we had an excellent one available to us.

Finally, and most importantly, this convergence of events included the arrival at Berkeley of Johan Wijler, a talented and disciplined young man who became my first graduate student in my new role as an assistant professor of plant biochemistry. He came to America as a refugee from Europe, with a sense of urgency to help solve the problems of the world and his fellowman. He was intense, quiet, and imaginative, with a keen sense of humor, and he was an excellent research companion. Together we explored denitrification in a complex system. (Unfortunately, within the decade, his life came to a tragic end.)

To avoid amplifier instability resulting from operation of the elevator, much of our mass spectrometer work, particularly when small enrichments were expected, was reserved for weekends when there was less activity in the building. Johan was a music lover; consequently the background sounds frequently included a barely audible classical concert on the radio over the rhythm of the vacuum pumps. Our samples were "blind" to discourage inadvertent bias, so the general atmosphere was one of relaxed suspense. Before a weekend was over, we frequently had new information, new questions, and new plans for the next week. Under these circumstances, one left the laboratory with reluctance, looking forward to the next experiment.

We were able to get a new understanding of the dynamic system we were working with by observing directly the influence of pH and other variables on intermediates and products of denitrification and their interconversion. We found the circumstances of nitric oxide formation and disappearance, fouling the mercury of the Toeppler pump in the process, and we learned many other unexpected and sometimes unwanted things.

Since this earlier work, more detail of the denitrification reaction has been revealed. In the past decade, attention has been focused on denitrification and nitrification as sources of atmospheric nitrous oxide.²

The source of some of the isotopically labeled nitrate ion was not discussed in this paper. Early enrichments of nitrogen were by countercurrent distillation of ammonia, and commercially labeled nitrate ion had to await the application of methods that used the oxides of nitrogen and its oxy-acids and related countercurrent processes.^{3,4} Methods in the literature for oxidizing ammonium ion to nitrate give low yields that discourage their application with valuable isotopically labeled ammonium ion. I obtained near-100 percent yields using enrichment cultures of the nitrifying microorganisms *Nitrosomonas* and *Nitrobacter*. This works because the energy yield in nitrification is sufficiently low that the organisms must process large quantities of their respective substrates for a comparatively small cell yield.

Much of the equipment we used was specialized and not available as ordinary catalog material. I was a somewhat shaky glassblower, and the line drawings in our paper, although otherwise accurate, probably showed the glassware as more symmetrical than it actually was. Not more than six months ago, under the banner of progress, I was cleaning out glassware cases in the laboratory and came upon some of the asymmetric apparatus I had made for that earlier study. Concluding that it probably would not be used again, I reluctantly consigned it to recycling. I could almost hear from the past the sound of the vacuum pumps and the warm, melancholy strains of Sibelius's *Valse Triste*.

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2. Delwiche C C, ed. *Denitrification, nitrification, and atmospheric nitrous oxide*. New York: Wiley, 1981. 286 p.
3. Spindel W & Taylor T I. Separation of nitrogen isotopes by chemical exchange between NO and HNO₃. *J. Chem. Phys.* 23:981-2, 1955.
4. ———. Concentration of nitrogen-15 by chemical exchange in a thermal diffusion column. *J. Chem. Phys.* 23:1318-22, 1955.