Reports

Nitrification in Paleocene Shale

Abstract. *Exchangeable ammonium nitrogen is present in Paleocene (Fort Union) shale below a depth of 10 meters in North Dakota and eastern Montana. Above 10 meters, exchangeable ammonium nitrogen is nitrified in situ. The lack of viable nitrifying organisms and the probable lack of oxygen prevent in situ nitrification below 10 meters. Shale samples incubated at 27°C under nonsterile conditions or shales exposed to atmospheric contamination exhibited active nitrification without additional treatment.*

Geochemists frequently report the total nitrogen content of sediments and sedimentary rocks. However, classical methods of fractionating nitrogen in sediments usually make it possible to identify only organic forms, providing little or no information on inorganic forms of nitrogen. Stevenson (*1*) was among the first to point out that as much as two-thirds of the total nitrogen in sediments and sedimentary rocks is entrapped in the lattice of clay minerals and is not measured by classical Kjeldahl methods. This entrapped nitrogen is mainly fixed NH_4^+ but may also include some nitrogen in similarly entrapped organic materials. The indigenous fixed NH_4^+ is a biologically inert form of nitrogen, becoming mobile primarily upon the destruction of the clay lattice (*2*).

More important biologically active forms of inorganic nitrogen include exchangeable NH_4^+ (ammonium ions adsorbed by cation exchange on the surfaces of clay particles) and water-soluble NO_2^- and NO_3^-. Information on these forms of inorganic nitrogen in either ancient or recent sediments is very limited. Stevenson (*3*) reported significant concentrations of exchangeable NH_4^+ in several shales of Paleozoic age. Van Schreven (*4*) has presented data on water-soluble, exchangeable, and fixed NH_4^+ in modern marine sediment from Lake Ijssel in the Netherlands; he also provided information on transformations of inorganic nitrogen upon incubation of samples of this sediment.

As part of a research program on the reclamation of lignite strip mine spoil piles in North Dakota and eastern Montana, we have studied the chemical forms and transformations of inorganic nitrogen in the beds of soft shale in the Fort Union group of Paleocene (early Tertiary) age. These shale beds are in the Tongue River and Sentinel Butte formations, frequently interbedded with lesser amounts of siltstone, sandstone, and lignite coal (*5*). Analyses of samples of these sedimentary rocks collected in 30-cm depth increments reveal that below 10 m the shale contains 300 to 500 parts per million (ppm) of total nitrogen by Kjeldahl analyses (*6*), 10 to 40 ppm of NH_4^+ nitrogen exchangeable with 2*N* KCl, 150 to 300 ppm of fixed NH_4^+ nitrogen released by HF treatment (*6*), and only 1 to 3 ppm of NO_3^- nitrogen (Fig. 1). The content of NO_2^- nitrogen is generally less than 1 ppm. From 2- to 10-m depths the maximum NO_3^- nitrogen concentrations are generally 20 to 40 ppm, but the concentration of exchangeable NH_4^+ nitrogen is only 2 to 5 ppm. In the upper 2 m, very little extractable inorganic nitrogen is present because of root absorption by higher plants. The concentration of fixed NH_4^+ in these upper strata (0 to 10 m) is similar to that in lower strata. This distribution of inorganic nitrogen is typical of that observed in shale at several mine sites that are located in the Fort Union region. The shale contains 40 to 70 percent montmorillonitic clay and up to 15 percent $CaCO_3$; in the solution extracted from a water-saturated slurry of shale (the saturation extract), the electrical conductivity is usually 1 to 3 mmho/cm at 25°C, the sodium adsorption ratio is 10 to 40, and the *p*H is 7.5 to 8.5 (*7*).

We observed that the exposure of shale from depths greater than 10 m to atmospheric weathering for one summer generally resulted in an almost complete reversal in the concentrations of exchangeable NH_4^+ and NO_3^- nitrogen. Typical values for freshly weathered shale are 2 ppm of exchange-

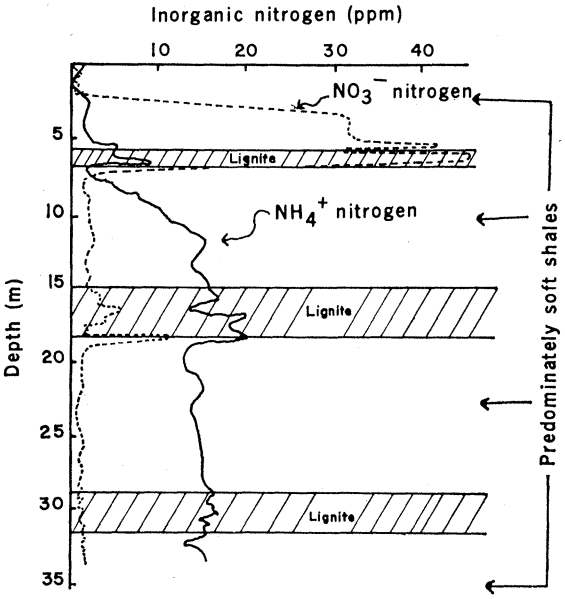

Fig. 1. Profile of the exchangeable NH_4^+ and NO_3^- nitrogen distribution with depth in undisturbed Fort Union sediments under native mixed prairie grasses.

Scoreboard for Reports: In the past few weeks the editors have received an average of 68 Reports per week and have accepted 12 (17 percent). We plan to accept about 12 reports per week for the next several weeks. In the selection of papers to be published we must deal with several factors: the number of good papers submitted, the number of accepted papers that have not yet been published, the balance of subjects, and length of individual papers.

Authors of Reports published in *Science* find that their results receive good attention from an interdisciplinary audience. Most contributors send us excellent papers that meet high scientific standards. We seek to publish papers on a wide range of subjects, but financial limitations restrict the number of Reports published to about 15 per week. Certain fields are overrepresented. In order to achieve better balance of content, the acceptance rate of items dealing with physical science will be greater than average.

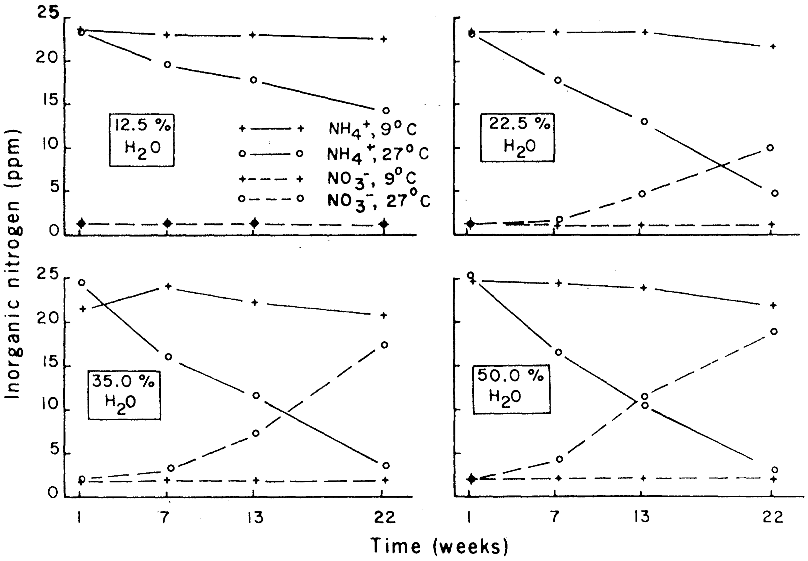

Fig. 2. Exchangeable NH_4^+ and NO_3^- nitrogen concentrations resulting from incubation at several temperatures and water contents.

able NH_4^+ nitrogen and 20 ppm of NO_3^- nitrogen. Frequently, exposure for an additional 1 to 2 years results in the disappearance of this NO_3^- nitrogen from the upper 30 cm of spoil material. The shale is of Paleocene age (70×10^6 years) and presumably lacked active nitrifying bacteria before disturbance. The absence of nitrifiers was confirmed by plate counts (*8*) made on unexposed samples collected by sterile techniques.

Nitrification of exchangeable NH_4^+ in situ at depths greater than 10 m is impossible, of course, in the absence of nitrifying organisms. Other factors required for nitrification are a carbon source, an oxygen supply, favorable ambient temperature, and a water supply (*9*). A readily available carbon source is the $CaCO_3$ in these shales (*7*). Lignite located near or at the surface is rapidly oxidized and unfit for use as a fossil fuel, but lignite beds more than 10 m below the surface are not oxidized, an indication that little or no oxygen has been available at this depth throughout the geologic history of the strata. Temperature profiles, measured with thermocouples, revealed that below about 10 m the ambient temperature is a constant 8.5°C throughout the year. The water content in situ of the shale is usually 20 to 25 percent (by weight), which is less than 25 percent of the water content at saturation, an indication of probable water tension in excess of 15 bars. Small quantities of free water are sometimes found in the lower part of shallow lignite beds, which frequently serve as the only source of domestic water even though the NO_3^- nitrogen content in this water is often in the 20- to 150-ppm range (*10*).

We conducted an incubation experiment to evaluate environmental conditions which favor nitrification of the exchangeable NH_4^+ in the shale. Shale samples used for this purpose were collected at a depth of approximately 18 m from a mine highwall about 4 weeks after the shale had been exposed to the atmosphere. These samples, inoculated with nitrifying organisms by atmospheric contamination, were incubated in 6-mil polyethylene plastic bags for 22 weeks at 9° or 27°C, and at water contents of approximately 12.5, 22.5, 35.0, or 50.0 percent (by weight). At 12.5 percent water, neither the exchangeable NH_4^+ nitrogen content nor the NO_3^- nitrogen content changed in 22 weeks in samples incubated at 9°C (Fig. 2), whereas at 27°C the NH_4^+ nitrogen content decreased somewhat without a corresponding change in the NO_3^- nitrogen content. At higher water contents, changes in the NH_4^+ and NO_3^- nitrogen concentrations of samples incubated at 9°C were relatively minor, whereas at 27°C the NH_4^+ nitrogen concentration notably decreased and the NO_3^- nitrogen concentration increased with time. The rate of change of these concentrations at 27°C increased with increasing water content.

These results indicate that exchangeable NH_4^+ nitrogen in the Paleocene shale is readily nitrified when moisture, temperature, and the oxygen supply are favorable. Restricted amounts of water or reduced temperatures may greatly reduce nitrification. Admittedly, a 22-week incubation period is much too short to permit any conjecture about the amount of nitrification that could have taken place over the 70×10^6 years since these sediments were deposited. Moreover, the region has probably undergone several drastic changes in climate since Paleocene time, suggesting that present-day ambient conditions may be of little value in assessing changes that occurred in the past.

Incubation did not result in a net increase in the inorganic nitrogen content. This fact suggests that the extent of mineralization of the small quantity of organic nitrogen present (about 200 ppm) in these sediments was minimal and indicates that this organic nitrogen is biologically relatively inert.

The results reported here show, first, that Fort Union (and presumably many other) sedimentary rocks contain appreciable quantities of exchangeable NH_4^+ nitrogen. Approximately 200 kg of exchangeable NH_4^+ nitrogen per hectare is initially present in each meter of thickness of these materials, an amount sufficient to meet the nitrogen requirements of most agricultural crops grown in the region for several years. Second, we found no viable nitrifying bacteria in the soft shale of Paleocene age until it was exposed to atmospheric contamination. This fact plus a probable lack of available oxygen effectively prohibits nitrification in situ at depths greater than 10 m. Third, the fact that our data show that nitrification of this exchangeable NH_4^+ nitrogen occurs readily after exposure to atmospheric conditions cautions us on the possible consequences of disturbing these materials. Strip mining or the irrigation of thousands of hectares of this shale annually greatly enhances the potential for a dangerous NO_3^- nitrogen buildup in surface water and groundwater and calls for well-designed and well-executed water-control practices. Conversely, bringing this shale to the surface does increase the available nitrogen and so may enhance the revegetation during reclamation of strip mine spoil piles, if properly handled.

J. F. Power, J. J. Bond
F. M. Sandoval, W. O. Willis
Northern Great Plains Research Center,
Agricultural Research Service,
U.S. Department of Agriculture,
Mandan, North Dakota 58554

References and Notes

1. F. J. Stevenson, *Science* **130**, 221 (1959).
2. H. Nommik, in *Soil Nitrogen*, W. V. Bartholomew and F. E. Clark, Eds. (Agronomy No. 10, American Society of Agronomy, Madison, Wisconsin, 1966), p. 198.

→ F. J. Stevenson, *Geochim. Cosmochim. Acta* **26**, 797 (1962).

4. D. A. van Schreven, *Plant Soil* **18**, 143, 163 (1963).
5. C. F. Royse, Jr., *Sediment. Geol.* **4**, 19 (1970).
6. J. M. Bremner, in *Methods of Soil Analysis*, part 2, C. A. Black, Ed. (American Society of Agronomy, Madison, Wisconsin, 1965), p. 1149; *ibid.*, p. 1179.
7. F. M. Sandoval, J. J. Bond, J. F. Power, W. O. Willis, *Proc. Mined-Land Reclamation Symp.* (Pittsburgh, Pa., 1973), p. 117.
8. M. Alexander and F. E. Clark, in *Methods of Soil Analysis*, part 2, C. A. Black, Ed. (Agronomy No. 9, American Society of Agronomy, Madison, Wisconsin, 1965), p. 1477. Bacterial counts were made by T. M. McCalla and his associates at the Agricultural Research Service, University of Nebraska, Lincoln.
9. M. Alexander, *Introduction to Soil Microbiology* (Wiley, New York, 1961), p. 275.
10. H. Trapp, Jr., *U.S. Geological Survey County Ground Water Studies 16*, part 2 (North Dakota State Water Commission, Bismarck, 1971), p. 416.

11. We thank T. M. McCalla and his co-workers for carrying out the bacterial counts.

30 November 1973; revised 4 January 1974 ■

Monolayers and Microbial Dispersal

Abstract. *Aqueous films on terrestrial litter are inhabited by numerous microorganisms; the surfaces of such films are covered by monolayer-forming substances. The spreading pressure of the latter can result in transport of floating and submerged organisms to adjacent water films with clean surfaces. The clean surfaces, produced by rain or possibly dew, permit rapid vertical and horizontal dispersal of microorganisms onto newly fallen leaves and other plant materials.*

During wet periods, a thin layer of water is present on leaves and other litter on the soil surface. This film is the habitat of numerous bacteria, protozoans, fungi, and other small organisms (*1*). The organisms inhabiting the film can be seen readily if moist litter is submerged in water in a glass dish and the air-water interface is examined microscopically (*2*). Among the more conspicuous objects often present are floating propagules (conidia) of various Fungi Imperfecti. While examining such conidia on water, we saw that the surface layer of water and floating objects often moved opposite the flow of the underlying water if the container was tilted. Furthermore, adjacent objects at the interface maintained a constant position with respect to one another, which suggests that the presence of monolayer substances at the air-water interface was responsible for the movement observed. These observations led to a series of experiments that demonstrated a previously undescribed mechanism for local dispersal of terrestrial microorganisms.

Initially, we used conidia from cultures of *Articulospora tetracladia*, *Cladosporium* sp., *Gyoerffyella (Ingoldia) craginiformis*, *Fusarium* sp., *Penicillium* sp., and *Varicosporium elodeae*, all of which had been isolated from the surface film from litter. Because of the masses of spores available, basidiospores of a puffball, *Lycoperdon perlatum*, spores of a myxomycete, *Fuligo septica*, and cells of bakers' yeast, *Saccharomyces cerevisiae*, were used in later experiments. With the exception of the yeast cells, all of these propagules float, either because of their form or because of hydrophobic surface layers. Masses of the spores were floated onto the surface of tap water in reservoirs (glass dishes). Clean glass strips 5.0 cm wide, 0.6 cm thick, and 1 m long were used to form inclined planes (Fig. 1), the lower ends of which terminated in the reservoirs with floating spores. In some experiments, the glass strips were used alone; in others, the strips served as support for either dialysis tubing or leaves. Distilled water (glass, distilled once) was allowed to drip slowly onto the upper end of the glass strip and to flow down the incline to the reservoir. In tests involving leaves, only senescent or recently fallen leaves of *Acer macrophyllum* were used; they were arranged shinglewise on the strip. Dialysis tubing was first wetted, then slipped over the glass strips and smoothed.

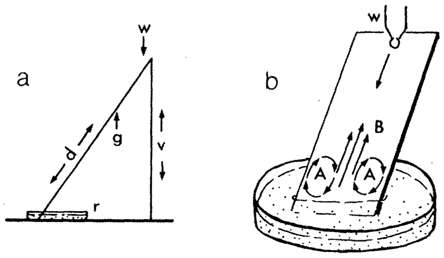

Fig. 1. (a) Diagram of experimental apparatus; *r*, reservoir with water and spores; *g*, glass strip; *w*, point of water application; *d*, distance from source traveled by spores; *v*, vertical distance traveled by spores. (b) Diagram showing surface motion of spores on water; *A*, rapid circulating motion; *B*, slow or rapid movement of spores toward water source at *w*.

As water flowed down the inclines, three different types of movement of the spores were observed: (i) rapid circulating movement from the reservoir surface onto the incline and back to the reservoir (*A* in Fig. 1); (ii) slow creeping movement of the spores up the wet surface of the incline (*B* in Fig. 1); and (iii) rapid movement of the spores onto the surface of the incline. These movements were, in part, correlated with rates of flow of water down the strip. The rapid circulating movements accompanied a high rate of flow; slow spread occurred while a very slow flow of water was maintained or after such a flow had been stopped; rapid spread occurred immediately after rapid flow was cut off. The greatest distances traveled by the spores were obtained when a very slow flow was maintained and then stopped. The distances are indicated in Table 1.

In the experiments described here, spores were transported equally well on clean glass strips, on dialysis membrane on such strips, and on leaf surfaces. No differences could be detected in distance of transport of differently shaped propagules; suspended yeast cells were transported to the same distances as floating propagules. When leaves were used, water was directed on the upper edge of the leaf and flow occurred mainly along the vein patterns. Both single leaves and laminations of several overlapping leaves were tested. The edges of laminated leaves did not present an insurmountable barrier to the upward transport of spores. However, it was found that living leaves, or such leaves pressed and dried, did not work well in these experiments. The intact cuticle prevented wetting of the surface.

Spores of *Fuligo* and *Lycoperdon* and yeast cells were used in large quantities; their transport could be followed visually. The distances traveled by other propagules used in the tests were determined by pressing microscope cover slips to the wet surfaces of leaves, then examining them microscopically. When glass strips alone or strips with dialysis membrane were used, the films were allowed to dry and the surfaces were then scanned with a microscope to determine the distances traveled.

In a second type of experiment, clean glass microscope slides or washed