Nitrification-Denitrification Reactions in Flooded Soils and Water Bottoms: Dependence on Oxygen Supply and Ammonium Diffusion

W. H. Patrick, Jr. and K. R. Reddy

ABSTRACT

Ammonium nitrogen in a flooded soil or water bottom exposed to oxygen from the water column undergoes sequential nitrification and denitrification. Oxygen moving through the overlying water column causes the development of an aerobic surface layer of soil or sediment. Ammonium in this aerobic surface layer is nitrified, and the resulting ammonium concentration gradient across the aerobic layer and the underlying anaerobic layer causes ammonium in the anaerobic layer to diffuse upward into the aerobic layer where it undergoes nitrification. Nitrate produced in the aerobic layer then diffuses downward into the anaerobic layer where it is denitrified to N₂ and N₂O. Nitrate derived from ammonium nitrogen in the aerobic layer appears as an intermediate product in the nitrification-denitrification reaction.

A laboratory experiment utilizing ¹⁵N as a tracer showed that approximately one-half of the nitrogen involved in the nitrification-denitrification process was ammonium originally present in the surface aerobic soil or water bottom layer with the remainder diffusing up from the underlying anaerobic layer. Where oxygen was absent or limiting, nitrification either did not occur or occurred at a lower rate, resulting in a reduced amount of nitrate available for the denitrification process.

Additional Index Words: N loss, ammonium oxidation, nitrate reduction, aerobic layer, anaerobic layer, submerged soil.

Denitrification in soils, swamps, marshes, and water bodies is the major process by which elemental nitrogen is returned to the atmosphere. Denitrification takes place when a deficiency of oxygen causes certain bacteria to use nitrate in place of oxygen as an electron acceptor for respiration. Although denitrification in soils as a result of temporary anaerobic conditions caused by variations in soil moisture has been recognized for years (e.g., Russell, 1961), the extent of and mechanisms involved in nitrogen loss in continuously flooded systems are not as well documented.

Ammonium is the predominant inorganic form of nitrogen in oxygen-deficient flooded systems and denitrification cannot take place unless conditions exist where nitrification of the ammonium can first occur. The development of a thin oxygenated surface layer as a result of dissolved oxygen penetrating a short distance into the flooded soil or sediment before being consumed allows the oxidation of ammonium to nitrate to take place in this oxygenated or aerobic surface layer. Nitrate produced from this reaction diffuses down into the underlying oxygen-free layer where it is denitrified.

Recent experiments have shown that appreciable denitrification will occur in flooded soils if both oxygen from the atmosphere and ammonium from the flooded soil are available (e.g., Tusneem and Patrick, 1971; Patrick and Tusneem, 1972; Broadbent and Tusneem, 1971; Patrick and Gotoh, 1974). Although the nitrogen converted from nitrate to gaseous forms in flooded systems is derived from ammonium oxidized to nitrate in the aerobic layer, the amount of nitrogen gas produced usually greatly exceeds the amounts of ammonium and nitrate present in the aerobic surface layer at any one time. Ammonium movement from the underlying anaerobic layer to the surface aerobic layer is apparently necessary to account for the large nitrogen losses that occur in flooded systems. Removal of ammonium by nitrification in the aerobic layer creates a concentration gradient which causes ammonium in the underlying anaerobic layer to diffuse upward to the aerobic layer where it undergoes nitrification. Nitrate formed by this process diffuses down toward the nitrate-free anaerobic layer where it is denitrified to nitrogen gas by serving as an electron acceptor in the oxidation of organic matter. These processes are illustrated in Fig. 1. As indicated in the equation in Fig. 1, nitrate is an intermediate product in the overall pathway between ammonium and elemental nitrogen.

In the experiments described in this report, the processes discussed above for governing nitrogen loss in flooded soils and water bottoms are examined. Particular attention is given to the roles of oxygen supply to the aerobic layer and ammonium diffusion from the anaerobic layer to the aerobic layer in determining nitrogen loss through the nitrification-denitrification process. Nitrogen-15-labelled ammonium was used to trace nitrogen through the various nitrogen forms, including the final gaseous form leaving the sediment.

MATERIALS AND METHODS

The soil used in the present study was Crowley silt loam which contained 0.702% total carbon, 0.081% total nitrogen, and had a pH of 5.5. The experiment was carried out using large (45.5 cm length by 4.5 cm diameter) pyrex tubes fitted at the top with a stopcock and a ground glass junction for entry to a mass spectrometer. All connections in the apparatus were ground glass and tested for leaks. Fifty grams of soil and enough water (50 mL) to provide a 1.5-cm water layer over the soil were added to each flask. This gave a 3.0 cm soil layer in the flask. Ammonium sulfate with a ¹⁵N enrichment of 29.944 atom-% was thoroughly mixed with the soil at the rate of 250 µg N/g soil. The effect of oxygen in the air over the flooded soil on the conversion of ammonium nitrogen to nitrogen gas was studied in two experiments. In one experiment, an atmosphere of 30% oxygen and 70% argon was maintained over the flooded soil. Duplicate flasks were removed after various periods of incubation for analyses of five labelled nitrogen fractions: ammonium, organic (protein) nitrogen, nitrate, nitrous oxide, and elemental nitrogen. The amount of labelled nitrogen was determined in each of these fractions by appropriate procedures employing a mass spectrometer (Bremer, 1965b; Cadz and Bartholomew, 1969). In the other experiment, conditions were the same as described above except that variable oxygen contents ranging from 0 to 50% were maintained over the flooded soil for 100 days, after which the distribution of labelled nitrogen was determined. Side tubes containing ascarite granules were placed in each flask to absorb excess carbon dioxide. The amount of oxygen consumed was also determined by measuring the amount of oxygen present initially and at the end of each incubation period in the incubation flask by means of a mass spectrometer. The size of the flask and

2 Professor and Research Associate, respectively.
Nitrification - Denitrification Reaction:

\[
24\text{NH}_4^+ + 48\text{O}_2 \rightarrow 24\text{NO}_3^- + 24\text{H}_2\text{O} + 48\text{H}^+
\]

\[
24\text{NO}_3^- + 5\text{C}_6\text{H}_5\text{O}_6 + 24\text{H}^+ \rightarrow 12\text{N}_2 + 30\text{CO}_2 + 42\text{H}_2\text{O}
\]

\[
24\text{NH}_4^+ + 5\text{C}_6\text{H}_5\text{O}_6 + 48\text{O}_2 \rightarrow 12\text{N}_2 + 30\text{CO}_2 + 66\text{H}_2\text{O} + 24\text{H}^+
\]

Fig. 1—Processes involved in the sequential conversion of organic nitrogen to elemental nitrogen in flooded soils and sediments. Ammonium is released from organic matter and diffuses upward into the aerobic soil layer where it is nitrified. Nitrate produced from this reaction diffuses down into the anaerobic layer where denitrification takes place. Oxygen diffusing into the soil from the water column creates an aerobic zone that is required for nitrification to occur.

When the amount of soil were chosen so that large changes in the oxygen content did not occur during incubation.

Separate experiments were carried out to measure the magnitude of ammonium diffusion from the anaerobic layer to the aerobic surface layer. One method consisted of incubating columns of flooded soil (20 cm long) containing 200 µg ammonium N/g soil and determining ammonium distribution at different times by slicing the soil columns horizontally into 2-mm sections. The sectioned samples were analyzed for ammonium by direct steam distillations (Brenner, 1965a). The distribution curves of ammonium in the aerobic and anaerobic layers were used to calculate the relative contribution of ammonium diffusion to N loss. The total shaded area in Fig. 2 represents the total ammonium N disappearance from the system during the 90-day incubation period, with the shaded portion of the aerobic layer representing that removed by nitrification and the shaded portion of the anaerobic layer representing the ammonium N loss due to diffusion from the aerobic layer to the aerobic layer, since nitrification does not occur in the anaerobic layer (Alexander, 1965). Another approach to the estimation of ammonium diffusion from the anaerobic layer into the aerobic layer was to measure the total amount of ammonium converted to nitrogen gas and nitrate and to subtract from this value the amount of ammonium originally present in the aerobic layer. Diffusion was not involved in the nitrification of ammonium originally present in the aerobic layer. In order to use this method it was necessary to allow the reaction to proceed long enough so that all of the ammonium originally present in the aerobic layer had been converted to nitrate. For both of the above methods, it was also necessary to accurately determine the thickness of the aerobic layer which was done by measuring the thickness of the reddish-brown, oxidized, ferric oxide layer overlying the grayish-brown, ferrous oxide layer. The thickness of the aerobic layer determined by this method was found to correspond closely with the thickness of the nitrate-containing layer.

RESULTS AND DISCUSSION

Results of the experiment dealing with the effect of oxygen on ammonium conversion to nitrate and then to nitrogen gas are shown in Fig. 3 and 4. The ammonium content of the system decreased with time with a buildup of nitrogen gas evident after 2 weeks. No nitrous oxide (N₂O) was present until the last sampling at 100 days. Nitrate was present after 30 days, but did not accumulate. The most striking result of this experiment was the rapid conversion of ammonium to nitrogen gas after 30 days. This length of time was approximately the same as that required for the development of a pronounced aerobic surface layer in the flooded soil. A small amount of labelled ammonium nitrogen was incorporated into the organic fraction at the beginning of incubation and did not change appreciably in concentration during the 100-day period.

The oxygen content of the atmosphere over the flooded soil during the 100-day incubation period had a marked effect on the stability of ammonium (Fig. 4). Where no oxygen was present almost all of the added labelled ammonium remained in the ammonium form except for the small amount incorporated into the organic fraction. Where oxygen was present, however, at least part of the ammonium was converted to nitrogen gas, with the
amount increasing as the oxygen content increased up to 26%. A higher oxygen content resulted in a thicker aerobic layer and slightly more nitrate, but no additional nitrogen gas.

Based on the thickness of the aerobic surface layer and the amount of labelled nitrogen gas produced, approximately twice as much ammonium was converted to nitrate and elemental nitrogen as was originally present in the aerobic layer. Calculation of ammonium diffusion from the anaerobic layer to the aerobic layer from direct measurement of ammonium distribution with depth throughout the aerobic-anaerobic layers also showed that approximately half of the ammonium nitrogen involved (500 µg/cm² or 50 kg/ha) was derived from ammonium diffusing from the anaerobic layer to the aerobic layer during the 90-day incubation period. The continuous disappearance of ammonium in the surface aerobic layer by nitrification established a concentration gradient across aerobic-anaerobic soil layers, which further enhanced the diffusion of ammonium from the anaerobic layer to the aerobic layer. The measured diffusion coefficient for ammonium moving in the saturated flooded Crowley silt loam was 2.5 × 10⁻⁴ cm²/sec as determined by the method of Phillips and Brown (1964). Much of the nitrate derived from the ammonium in the aerobic layer diffused back into the anaerobic layer, because of a nitrate concentration gradient established across aerobic and anaerobic soil layers. Nitrate reaching the anaerobic layer was very unstable and was rapidly denitrified to nitrous gas. Rapid movement of nitrate from the aerobic layer to the anaerobic layer prevented accumulation of nitrate in the aerobic layer. The measured diffusion coefficient for nitrate moving in a water-saturated soil was 1.3 × 10⁻⁵ cm²/sec, which is about sixfold greater than ammonium diffusion (Reddy et al., 1975). These results are applicable for flooded soil or sediment systems in which no downward percolation occurs that could prevent upward diffusion of ammonium. In most flooded soils and shallow water bodies percolation of water is very slow.

Oxygen consumption in the flooded soil is shown in Fig. 5. Most of the oxygen consumed initially was probably used for oxidation of organic carbon by heterotrophs, but after several days a considerable portion was also being used for nitrification. Some of this oxygen was present as nitrate at the time of measurement, while some had been converted from nitrate to carbon dioxide in the denitrification reaction (see equation in Fig. 1). Increasing the concentration of oxygen over the flooded soil increased the thickness of the aerobic layer and increased the amount of oxygen consumed. Nitrification and organic carbon oxidation accounted for approximately equal amounts of oxygen.

The results of this study show that ammonium nitrogen in an anaerobic soil or sediment exposed to atmospheric oxygen undergoes sequential nitrification and denitrification. The source of the ammonium reacting biologically with atmospheric oxygen in the aerobic layer to produce nitrate consisted of the ammonium initially present in the aerobic surface layer of soil or sediment plus an approximately equal amount diffusing to the aerobic layer from the underlying anaerobic layer. It is likely that a significant part of the ammonium present in flooded soils and the sediments underlying oxygenated water columns follows this pathway and makes a major contribution to the elemental nitrogen being returned to the atmosphere.

**LITERATURE CITED**


