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# Variation in Heterotrophic and Autotrophic Nitrifier Populations in Relation to Nitrification in Organic Soils†

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The occurrence of heterotrophic and autotrophic nitrifiers in Pahokee muck and the role of these organisms in the ecosystem were assessed by surveying their population densities under different field conditions and by observing the relationship of these populations with aerobic bacteria and soil moisture. Heterotrophic nitrifier populations varied from $2.0 \times 10^5$ to $3.8 \times 10^6$ bacteria per $cm^3$ of muck in surface fallow (bare) Pahokee muck during the annual cycle. This population decreased 40-fold between the surface and the 60- to 70-cm depths of soil. Similar variations were noted with autotrophic nitrifier populations. Significant correlations were found between heterotrophic nitrifiers and both soil moisture and aerobic bacteria. These relationships did not exist for the autotrophic nitrifiers. In soil that had been heated to kill the autotrophic nitrifiers, while preserving a population of the heterotrophs, and then amended with sodium acetate or ammonium sulfate or both, no nitrate or nitrite accumulated, although significant increases in heterotrophic nitrifiers were detected. In unheated control soil, nitrate plus nitrite-N increased from 14.3 to 181 μg/g of wet soil, and 48 μg of nitrite-N per g was produced. These data suggest that the autotrophic nitrifiers were the sole population responsible for nitrification in Pahokee muck.

Although heterotrophic nitrifiers have been shown to occur in several ecosystems (5, 6, 7, 10–12, 14), their ability to oxidize reduced nitrogenous compounds in environments other than axenic laboratory cultures has been questioned. The problem in assigning a definitive function to this group of organisms arises from the observations that the oxidation of nitrogen is not obligatorily linked to growth and, in pure culture, the production of nitrate or nitrite or both occurs after the growth phase (3, 9). Neither observation, however, precludes the function of these organisms in nitrite and nitrate production in natural systems. A suggested role for the heterotrophic nitrifiers was provided by Verstraete and Alexander (14) when they demonstrated the presence of hydroxylamine, 1-nitrosoethanol, nitrite, and nitrate (products of heterotrophic nitrification) in sewage, soil, and lake water amended with both ammonium and a carbon source. The carbon source was needed for this nitrification to occur.

The occurrence of large populations of heterotrophic nitrifiers in organic soils (Histosols) was recently demonstrated (12). These studies with ammonium- and acetate-amended Pahokee muck and with the nitrification inhibitor N-Serve [2-chloro-6-(trichloromethyl)pyridine] (Dow Chemical Corp., Midland, Mich.) suggested that the heterotrophs may produce some of the nitrate found in the organic soils. Because of the large populations of heterotrophic nitrifiers found in these soils and their suggested role in nitrification, it was considered that Pahokee muck would provide an ideal medium for further study of the function of these interesting organisms in a natural ecosystem. Thus, the effect of environmental parameters on the population densities of autotrophs and heterotrophs was determined to note if the two nitrifier populations varied similarly under like conditions and to document the extent of occurrence of heterotrophic nitrifiers in Histosols. Also, population changes and the production of nitrate and nitrite in Pahokee muck treated to inactivate autotrophs, but not heterotrophs, were examined.

## MATERIALS AND METHODS

The soil used in this study was Pahokee muck (a lithic medisaprist) collected at the Agricultural Research and Education Center at Belle Glade, Fla. For determining variation in microbial activity, soil samples were collected as follows: surface (0- to 10-cm) samples were composite samples from a 5-ha fallow (bare) field, an adjacent field of St. Augustine grass [*Stenotaphrum secundatum* (Walt) Kuntz], and a nearby sugarcane (*Saccharum* spp. L.) field. Soils within the profile were collected by digging a trench (2 by 2 m) in the fallow field to the indicated depth. Composite samples were collected at several depths. All soil samples were placed in sterile plastic bags and

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transported to the laboratory (transit time, approximately 5 min), where they were diluted in water for microbial analysis.

Heterotrophic nitrifiers were assayed as previously described (12). For this, the appropriate soil dilutions were plated on soil extract agar by the spread plate technique. After 7 days of incubation at 30°C, the numerically dominant colonies were selected and tested for nitrification capacity in a broth developed by Eylar and Schmidt (7). Autotrophic nitrifiers were assayed by the method of Alexander and Clark (2), except that a 35-day incubation period was used instead of the 21-day period. Aerobic bacterial populations were estimated by plating appropriate soil dilutions on soil extract agar (12). Soil moisture was determined by drying samples at 110°C to a constant weight. Values are presented as the percentage of the wet weight of soil.

For those studies requiring the amendment of the soil in the laboratory, fallow surface soil, which had been sieved to 2 mm, was dispensed into milk dilution bottles in samples of 10 g per bottle. To inactivate the autotrophic nitrifiers, the soil was heated at 100°C in an oven for 16 h. After the soil had cooled to room temperature, the soil moisture was adjusted to approximately 60%, and the soil was incubated for 7 days at 25°C to allow the surviving populations to equilibrate. After 7 days, sterile sodium acetate or $(NH_4)_2SO_4$ or both were added to give final concentrations of 1.0% (wt/wt) and 0.94% (wt/wt) (100 μg of N per g of soil), respectively. Microbial populations were assayed as indicated above. Ammonium and nitrate plus nitrite concentrations were estimated by titration after steam distillation of KCl extracts by the method of Bremner and Keeney (4). Nitrite was estimated in the extracts by the colorimetric method of Montgomery and Dymock (8).

## RESULTS

Initial studies involved the examination of the variation of the population densities of both heterotrophic and autotrophic nitrifying populations and aerobic bacteria in Pahokee muck with time, crop, and soil moisture. Heterotrophic nitrifier populations ranged from a minimum of $2.0 \times 10^5$ to a maximum of $3.9 \times 10^6$ bacteria per $cm^3$ of muck in the fallow surface (0- to 10-cm) soil between December 1976 and February 1978 (Fig. 1). This population fluctuated in like manner as soil moisture and aerobic bacterial populations. When examined by linear regression analysis, the relationship of heterotrophic nitrifiers to soil moisture indicated that in the moisture range detected (40 to 60%), increased moisture stimulated the heterotrophic nitrifier population. The correlation of the two variables was statistically significant (Table 1). Within the soil profile, as the depth of soil increased, the number of heterotrophic nitrifiers per cubic centimeter of soil declined (Fig. 2). With the soil profile samples, the moisture ranged from approximately 55% to saturation. At the higher

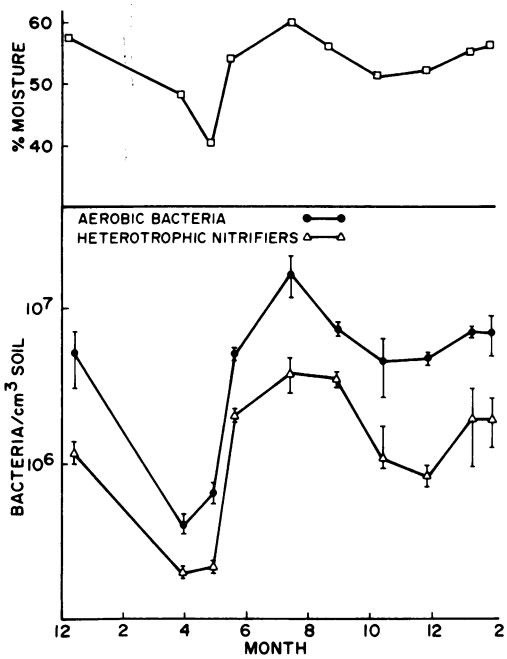


FIG. 1. *Variation of aerobic bacteria, heterotrophic nitrifiers, and percent moisture in surface (0- to 10-cm) fallow Pahokee muck from December 1976 to February 1978.*

TABLE 1. *Comparison of the relationship between autotrophic and heterotrophic nitrifiers and soil moisture and aerobic bacteria by linear regression analysis*

| Sample type | x | y | Correlation coefficient |
|---|---|---|---|
| Fallow surface soil | Het. Nitr.[a] | $H_2O$ | 0.76[c] |
| | Nitrite oxidizers | $H_2O$ | 0.16 |
| | Ammonium oxidizers | $H_2O$ | 0.34 |
| | Het. Nitr. | Aerob. Bact.[b] | 0.79[c] |
| Soil profile | Het. Nitr. | $H_2O$ | −0.53[c] |
| | Nitrite oxidizers | $H_2O$ | −0.25 |
| | Ammonium oxidizers | $H_2O$ | 0.21 |
| | Het. Nitr. | Aerob. Bact. | 0.91[c] |
| Surface soils | Het. Nitr. | Aerob. Bact. | 0.83[c] |

[a] Het. Nitr., heterotrophic nitrifiers.
[b] Aerob. Bact., aerobic bacteria.
[c] Correlation significant at the 0.99 level.

moisture levels, the resultant exclusion of air from the soil caused an inhibition of aerobic microbial activity (13). Hence, the negative correlation coefficient indicated that although there was a significant correlation, the two variables were related inversely. Significant variation in both autotrophic and heterotrophic nitrifiers was noted between the cropped soils and the fallow soils (Table 2). In each of the environments examined, the heterotrophic nitrifiers varied in parallel with the aerobic bacterial populations. Linear regression analysis of the relationship between these populations indicated a correlation that was significant, in all cases, at the 0.99 level (Table 1).

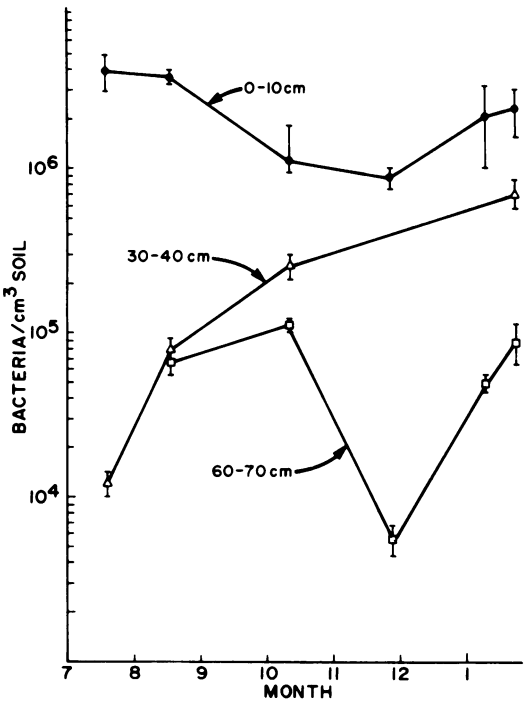


FIG. 2. *Variation of heterotrophic nitrifiers with depth of muck from July 1977 to February 1978.*

Changes in the population densities of the autotrophic nitrifiers appeared to follow the same pattern as did those of the heterotrophic organisms. For example, with the 8 January sample, a 40-fold decrease in heterotrophic nitrifiers occurred between the surface and the 60-cm depth, whereas the ammonia oxidizer and nitrite oxidizer populations declined from $2.0 \times 10^3$ to $7.7 \times 10/cm^3$ and $1.8 \times 10^4$ to $1.5 \times 10^2/cm^3$, respectively (Table 3). Similar variations were recorded for the other sample periods. Comparison of these populations with soil moisture levels revealed no significant correlation (Table 1). This suggests that in opposition to the situation with the heterotrophic nitrifiers, factors other than soil moisture were the primary controllers of the autotrophic population density. Thus, it appears that the two populations (autotrophic versus heterotrophic nitrifiers) were limited by different environmental parameters in Pahokee muck.

An ideal study of the role of heterotrophic nitrifiers would be to examine nitrogen oxidation by these organisms in the absence of autotrophic nitrifiers. No natural samples were found with one, but not the other, population. Thus, to create such a soil sample, fallow surface Pahokee muck was collected and heated as indicated

TABLE 2. *Nitrifiers in fallow, cane, and St. Augustine grass surface (0- to 10-cm) soils*

| Date[a] | Soil | Ammonia oxidizers[b] (MPN/cm³ of soil) | Nitrite oxidizers[b] (MPN/cm³ of soil) | Heterotrophic nitrifiers[c] (bacteria/cm³ of soil) |
|---|---|---|---|---|
| 3-29-77 | fallow | $7.5 \times 10^3$ CD | $3.6 \times 10^4$ CD | $(1.9 \pm 0.2) \times 10^5$ |
| | grass | $9.4 \times 10^3$ CD | $2.3 \times 10^4$ CD | $(5.4 \pm 0.7) \times 10^5$ |
| | cane | $1.7 \times 10^4$ CD | $3.9 \times 10^4$ CD | $(9.2 \pm 1.7) \times 10^4$ |
| 5-17-77 | fallow | $10^4$ CD | $1.9 \times 10^4$ D | $(2.1 \pm 0.07) \times 10^6$ |
| | grass | $7.4 \times 10^4$ A | $3.3 \times 10^4$ CD | $(1.6 \pm 0.3) \times 10^7$ |
| | cane | $4.9 \times 10^4$ AB | $4.6 \times 10^3$ E | $(2.2 \pm 0.4) \times 10^6$ |
| 1-18-78 | fallow | $4.2 \times 10^4$ AB | $3.9 \times 10^4$ CD | $(2.0 \pm 1.1) \times 10^6$ |
| | grass | $10^5$ A | $1.4 \times 10^4$ D | $(1.5 \pm 0.3) \times 10^6$ |
| | cane | $4.9 \times 10^4$ AB | $3.5 \times 10^4$ CD | $(4.2 \pm 2.1) \times 10^6$ |
| 2-8-78 | fallow | $4.6 \times 10^3$ D | $6.5 \times 10^4$ BC | $(2.9 \pm 0.7) \times 10^6$ |
| | grass | $2.1 \times 10^4$ BC | $6.4 \times 10^5$ B | $(2.3 \pm 1.3) \times 10^6$ |
| | cane | $2.2 \times 10^4$ BC | $1.4 \times 10^5$ A | $(2.6 \pm 0.3) \times 10^6$ |

[a] Month-day-year.

[b] Most probable number (MPN) values within a genus followed by a different letter are significantly different at the 95% level (1).

[c] Population ± standard error of the mean ($n = 3$).

above. The control soil was not heated. The heat treatment resulted in the reduction of the autotrophic nitrifier populations to undetectable levels while maintaining a population of approximately $1.3 \times 10^6$ heterotrophic nitrifiers per g of wet soil (Table 4). No detailed speciation was conducted on the surviving heterotrophic nitrifiers, but it was noted that a minimum of seven colonial types were preserved. During the incubation of the acetate- or ammonium-amended heated soil or both, although a significant increase in heterotrophic nitrifiers occurred (Table 4), no significant increase in nitrate plus nitrite concentrations was observed (Fig. 3A and B). Nitrite was not detected in these samples. In the control soil which contained both autotrophic and heterotrophic nitrifiers, ammonium amendment resulted in increases in nitrate plus nitrite-N levels from 14.3 to 181 μg/g of wet soil (Fig. 3C). Approximately 48 μg of nitrite-N per g was also produced in the latter samples. This nitrogen oxidation resulted in 800- and 600-fold increases in ammonia oxidizer and nitrite oxidizer populations, respectively (Table 4). These data suggest that the production of both nitrate and nitrite in Pahokee muck resulted from the metabolism of the autotrophs.

TABLE 3. *Variation of autotrophic nitrifiers with depth of muck*

| Date[a] | Depth (cm) | Moisture (%) | Ammonia oxidizers[b] (MPN/cm³ of soil) | Nitrite oxidizers[b] (MPN/cm³ of soil) |
|---|---|---|---|---|
| 12-20-77 | 0–10 | 55.6 | $6.8 \times 10^3$ A | $3.1 \times 10^5$ A |
| | 30–40 | ND[c] | ND | ND |
| | 60–70 | 81.2 | $<10^2$ | $<10^2$ |
| 1-8-78 | 0–10 | 55.4 | $2.0 \times 10^3$ B | $1.8 \times 10^4$ B |
| | 30–40 | ND | ND | ND |
| | 60–70 | 85.5 | $7.7 \times 10$ D | $1.5 \times 10^2$ D |
| 2-8-78 | 0–10 | 57.5 | $2.1 \times 10^3$ A | $2.2 \times 10^4$ B |
| | 30–40 | 80.2 | $2.7 \times 10^2$ C | $3.3 \times 10^3$ C |
| | 60–70 | 80.3 | $2.6 \times 10$ D | $5.2 \times 10^2$ C |
| 4-14-78 | 0–10 | 48.3 | $7.1 \times 10^2$ BC | $4.9 \times 10^4$ B |
| | 30–40 | 81.2 | $4.6 \times 10$ D | $1.5 \times 10^3$ C |
| | 60–70 | 81.7 | $<10^2$ | $6.0 \times 10$ D |

[a] Month-day-year.
[b] Most probable number (MPN) values within a genus followed by a different letter are significantly different at the 95% level (1).
[c] ND, Not determined.

## DISCUSSION

This study documents the extensive occurrence of heterotrophic nitrifiers in Pahokee muck. Definitive evidence for the function of these nitrifiers in natural ecosystems is difficult, if not impossible, to obtain. Although neither of the studies presented here is a direct approach to the question, they do strongly indicate that if the heterotrophs are functional in the oxidation of nitrogen in Histosols, their role is minimal. Comparison of the relationship of the population densities of nitrifiers to soil moisture indicated that this variable affected the two populations

TABLE 4. *Variation in bacterial populations per gram of moist soil in heated soil plus acetate or ammonium or both and freshly collected soil plus ammonium*

| Soil | Day | Aerobic bacteria[a] ($\times 10^6$) | No. of nitrifiers | | |
|---|---|---|---|---|---|
| | | | Heterotrophic[a] ($\times 10^5$) | Ammonia oxidizers[b] | Nitrite oxidizers[b] |
| Heated + N | 0 | 8.4 B | 13 B | ND[c] | ND |
| | 4 | 6.2 B | 19 AB | ND | ND |
| | 7 | 9.2 B | 7.3 C | ND | ND |
| | 9 | 33 A | 26 A | ND | ND |
| | 11 | —[d] | — | ND | ND |
| Heated + N + C | 0 | 9.4 A | 14 B | ND | ND |
| | 4 | 15 A | 36 A | ND | ND |
| | 7 | 17 A | 41 A | ND | ND |
| | 9 | 10 A | 14 B | ND | ND |
| | 11 | — | — | ND | ND |
| Unheated + N | 0 | 34 A | 99 A | $1.3 \times 10^3$ B | $4.0 \times 10^3$ D |
| | 4 | 13 C | 65 B | $3.0 \times 10^5$ A | $1.8 \times 10^4$ C |
| | 7 | 23 B | 55 C | $6.8 \times 10^5$ A | $6.6 \times 10^4$ B |
| | 9 | 7.3 D | 11 D | $5.1 \times 10^5$ A | $6.4 \times 10^4$ B |
| | 11 | — | — | $8.4 \times 10^5$ A | $2.6 \times 10^5$ A |

[a] Values followed by a different letter are significantly different at the 95% level (Duncan's new multiple range test).
[b] Values followed by a different letter are significantly different at the 95% level (1).
[c] ND, None detected.
[d] —, Not determined.

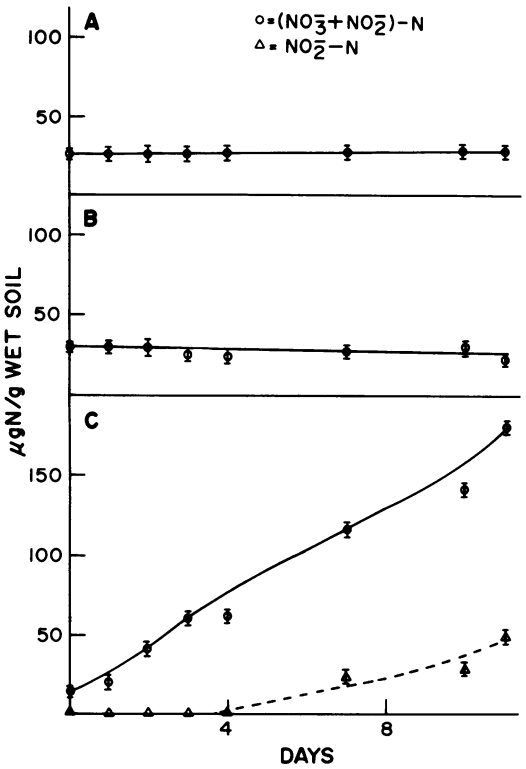


FIG. 3. *Oxidation of ammonium in heat-shocked Pahokee muck amended with sodium acetate or $(NH_4)_2SO_4$ or both and unheated Pahokee muck amended with $(NH_4)_2SO_4$. (A) Heated soil plus $(NH_4)_2SO_4$. (B) Heated soil plus sodium acetate and $(NH_4)_2SO_4$. (C) Unheated soil plus $(NH_4)_2SO_4$.*

differently. In fact, the relationship of heterotrophic nitrifiers to aerobic bacterial population densities suggests that variation of the heterotrophic nitrifiers was directly related to processes that stimulate heterotrophs in general and not to factors controlling nitrification specifically. This hypothesis was supported by population changes in the artificial ecosystem created by heating the soil samples. Although large populations of heterotrophic nitrifiers survived the high temperatures, no nitrification occurred after amendment of the heated soil with acetate or ammonium or both, but the population of heterotrophic nitrifiers increased. These two studies combined suggest that the sole population involved in the production of oxidized nitrogen in Pahokee muck is that of the autotrophic nitrifier.

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