NITRIFICATION PROCESS IN TEA FIELD SOILS OF JAPAN

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Introduction

Soil pH is a limiting factor for nitrification. The nitrification activity is higher in neutral or slightly alkaline soils. The nitrification potential has been found to be negligible below pH 5.0 and the lowest value of pH is considered to be around 4.5 (Dancer et al. 1973; Gilmour 1984).

On the other hand, some investigations indicated that nitrification can occur in strongly acidic soils with pH values of 4 to 5 (Weber and Gainey 1962; Weier and Gilliam 1986). It was suggested that heterotrophic nitrification was responsible for the formation of nitrite or nitrate in acid soils (Focht and Verstraete 1977). However, the process of nitrification in acid soils is poorly understood.

The soil pH of tea fields becomes strongly acid by heavy fertilization. However nitrification can occur in tea soils despite a low pH value of about 4. We used the soils of a tea field to investigate the nitrification process under acidic soil conditions.

Lowest value of pH for intrification in tea soil

Twelve soil samples were collected from a tea field in Japan. The pH of the samples ranged from 3.3 to 5.1, organic carbon content from 1.1 to 11.6% and total nitrogen content from 0.1 to 0.9%. The nitrification rate was measured by the soil incubation method. The nitrification activity showed a positive correlation, r=0.81 (1%significance) with the soil pH. The relation suggested that the nitrification activity was zero at around pH 2.9. This value is very low since pH 4.5 had been considered to be the lowest limit by other investigators.

Characteristics of nitrification in tea field soils

Two soil samples (red and yellow soil; pH 3.6, andosol; pH 3.5) were used to evaluate the characteristics of nitrification in the tea soils. The nitrification activity was measured by the soil incubation method. [1]

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Nitrapyrin which is considered to inhibit the ammonia oxidation system, was used as selective inhibitor in order to differentiate autotrophic from heterotrophic nitrification (Shattuk and Alexander, 1963). Nitrification in the tested soils was completely inhibited by the nitrapyrin treatment, indicating that nifrification in the tea field soils was caused by autotrophic ammonia-oxidizing bacteria.

The optimum temperature of the nitrification activity in the tea soils ranged between 25 and 30°C (Figure 1). Nitrification was very slow at 0°C. The rate decreased rapidly beyond 30°C and nitrification ceased at 40°C. The soil samples were treated with $(NH_4)_2SO_4$ at various levels to determine the effect of the NH_4-N concentration on the nitrification activity. The nitrification activity increased with increasing NH_4-N concentration from 5 to 30 mgN/100g of soil, but no significant change occurred with further increase of the NH_4-N concentration from 30 to 100 mgN. The rate of nitrification decreased with the increase of the NH_4^+-N concentration from 200 to 300 mgN. The maximum acceptable concentration of NH_4^+-N in our study was above 100 mgN/100g, a value higher than that reported in other observations (Harada and Kai 1968; Malhi and McGill 1982). These results indicate that the nitrifying bacteria in the tea field soils were tolerant to high NH_4^+-N and salt concentrations.

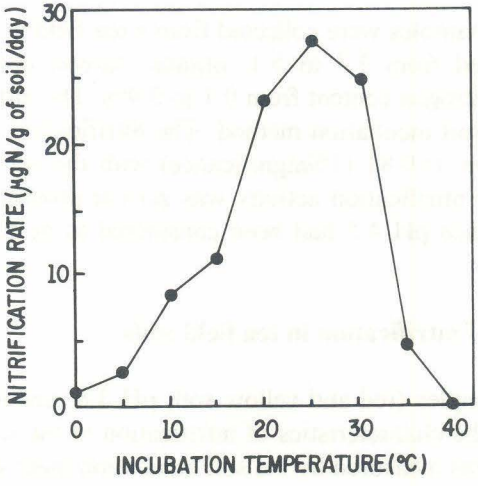

FIG. 1 - Effect of temperature on nitrification activity soil sample: Andosol, pH(H_2O) = 3.5.

Various levels of $CaCO_3$ were added to the soils to study the effect of the pH on the nitrification activity in tea field soils. Ammonia oxidation was not affected by the addition of $CaCO_3$, and proceeded very rapidly in all the soils regardless of the adition of $CaCO_3$. It appeared that the nitrification process in tea field soils was associated with the activity of autotrophic nitrifying bacteria that had adapted to acidic conditions.

The soil incubation method was used to observe the effect of nitrification on soil acidification. After the addition of 20,50 and 100 mg N of $(NH_4)_2SO_4$/100g of soil, almost all of $NH_4^+ - N$ was oxidized within 25 days (Figure 2). The pH of the soil samples decreased from the initial values of 4.3 at 50 mgN and 4.2 at 100 mg to final values of 3.5 and 3.0, respectively (Table 1). These findings indicated that nitrification is a major factor for soil acidification.

TABLE 1 - Change of soil pH after the addition of $(NH_4)_2SO_4$ and incubation at 25°C.

$NH_4^+ - N$ Added	Soil pH				
	Incubation time (days)				
(mgN/100g soil	0	5	11	18	26
20	4.4	4.3	4.1	4.0	3.9
50	4.3	4.2	3.8	3.5	3.5
100	4.2	4.1	3.6	3.1	3.0

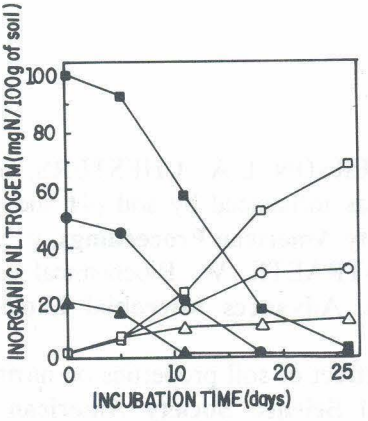

FIG 2 - Changes of inorganic N level after addtition of $(NH_4)_2SO_4$

○△□: NO_3-N ●△■: NH_4-N △△: 20 mgN of $(NH_4)_2SO_4$/100 g of soil
○●: 50 mgN of $(NH_4)_2SO_4$/100 g of soil □■: 100 mgN of $(NH_4)_2SO_4$/100 g of soil

Isolation of acidophilic ammonia-oxidizing bacteria

The most probable number technique was applied to isolate autotrophic ammonia-oxidizing bacteria as described by Schmidt and Belser (1982).

Two types of autotrophic ammonia-oxidizing bacteria were isolated from tea field soils. One of the isolates was acidophilic and the other was neutrophilic. The acidophilic ammonia-oxidizing bacteria were assigned to the *Nitrosococcus genus* based on morphological characteristics. The optimum pH for growth was around pH 5 in pure culture. Autotrophic ammonia-oxidizing bacteria have been considered to be neutrophilic and the lowest pH value compatible with growth was approximately pH 6.5 in pure culture (Watson 1974). Our results indicated that nitrification in acidic tea soils can be attributed to the activity of acidophilic ammonia-oxidizing bacteria.

Conclusion

1. The lowest value of pH for nitrification in tea field soils was approximately pH 2.9.
2. Nitrification was a major factor for the acidification of tea field soils.
3. Nitrification in the tea soils could be attributed to the activity of acidophilic ammonia-oxidizing bacteria.

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