

## Factors Affecting Nitrification in Soils

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**Abstract:** Nitrification in soil converts relatively immobile ammonium-nitrogen (N) to highly mobile nitrate-N (via nitrite), and this has implications for N-use efficiency by agricultural systems as well as for environmental quality, especially in situations where the potential for loss of soil or added N is high following nitrate formation. The literature on various physical, environmental, and chemical factors and their interactions on nitrification in soil is reviewed and discussed with examples from natural and agro-ecosystems. Among the various factors, soil matrix, water status, aeration, temperature, and pH have strong influence on nitrification. The information on factors that influence nitrification is useful when developing strategies for regulating nitrification in soils by employing chemical or biological nitrification inhibitors.

**Keywords:** Control of nitrification, N and environmental quality, N loss following nitrification, nitrification and N-use efficiency, nitrification inhibition, and physical, environmental, and chemical factors and nitrification

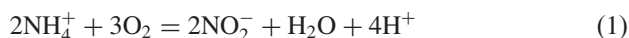
### INTRODUCTION

The biological oxidation of ammonium ( $\text{NH}_4^+$ ) or ammonia ( $\text{NH}_3$ ) to nitrate is known as nitrification. The nitrification of ammonium in soils is a two-step process in which ammonium or ammonia is first converted to nitrite ( $\text{NO}_2^-$ ) and then to nitrate ( $\text{NO}_3^-$ ). The conversion to nitrite is brought about largely

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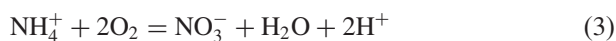
by a group of obligate autotrophic bacteria known as *nitrosomonas* by the following reaction:



The conversion from nitrite to nitrate is effected largely by a second group of obligate autotrophic bacteria termed *nitrobacter*. The reaction may be represented by the following equation:



The overall nitrification process is represented by the following equation:



*Nitrosomonas* and *nitrobacter* group of bacteria are collectively referred to as the *nitrobacteria* (Prosser 1989).

Also, a few heterotrophs have been reported to carry out nitrification in soil, but usually at much lower rates than those by autotrophic bacteria. The heterotrophic bacteria may contribute to nitrification in soils under harsh conditions such as those found in acid soils (Focht and Verstraete, 1977; Duggin, Voigt, and Bormann 1991; De Boer and Kowalchuk 2001).

Nitrification converts a relatively immobile ammonium-nitrogen (N) to highly mobile nitrate-N, and this has implications not only for N-use efficiency for crop production but also for environmental quality (Sahrawat 1989; Subbarao et al. 2006).

The nitrification reaction releases hydrogen ( $\text{H}^+$ ), which results in acidification of the soil when ammoniacal and most organic N fertilizers are converted to nitrate (Tisdale and Nelson 1970).

For nitrification to take place, optimum conditions in terms population of nitrifying organisms, pH, temperature, oxygen, moisture, and substrate concentration and availability are most important. At the ecosystem level, several physical, environmental, and chemical factors interact in a complex manner to influence the nitrification process.

The objective of this article is to review various physical, environmental, and chemical factors that affect soil nitrification, with examples from natural and agro-ecosystems. A better understanding of factors that influence nitrification in soils helps when controlling or regulating nitrification in the research effort toward increasing N-use efficiency and maintaining environmental quality, especially in situations where the loss of soil and applied N following nitrification is high (Sahrawat 1989; Subbarao et al. 2006).

## FACTORS INFLUENCING NITRIFICATION IN SOILS

### Physical Factors

Among the physical factors that influence nitrification, the availability or accessibility of the substrate (ammonium) to the nitrifying bacteria is crucial. The availability of substrate can be limited by protection of N from microbial attack through sorption or fixation of organic N and ammonium ions by soil clay minerals and by biological immobilization of ammonium (Focht and Verstraete 1977; Sahrawat 1979; Baldock and Skjemstad 2000; Johnson, Cheng and Burke 2000; Wang et al. 2003).

Strong, Sale, and Helyar (1999) studied the influence of the soil matrix on N mineralization and nitrification in small, undisturbed soil volumes. The soil volumes were treated with clover-derived substrate, dried and rewetted. The soil volumes were then incubated for 20 days at a matric water potential of either  $-10$  or  $-30$  kPa. Regression analysis of the data showed that clay and sand had a significant influence on nitrification, but silt did not. In soils retaining continuous moisture, percentage of clay had a negative relationship with nitrification, but this relationship was positive in soils that had been dried and rewetted. The results suggest that during periods of relatively high moisture content, soils that are higher in clay are able to protect organic N more effectively from microbial attack. On drying and rewetting, the protective mechanisms of clay are undermined, and large amounts of organic N become available for mineralization and subsequent nitrification. Soils higher in clay experience a greater flush of mineralization and nitrification than soils with lower clay content. Clay also influenced N mineralization and nitrification by limiting the diffusion of partially decomposed organics. However, texture had very little influence on the nitrification of urea-derived ammonium-N (Strong, Sale and Helyar 1999).

The interaction between moisture and aeration (oxygen status) relationships in the soil matrix greatly influences the nitrification, nitrate formation, and its stability. Under higher soil moisture, most of the soil pore spaces are occupied by water, and this negatively affects soil aeration and nitrification. Another important factor that influences nitrification is the presence of surfaces, especially in nutrient-poor environments, because surfaces enhance the establishment of microbial biomass by providing attachment sites and by concentrating nutrients from dilute solution (Focht and Verstraete 1977).

### Environmental Factors

Among the environmental factors that influence nitrification in soil, aeration (oxygen), temperature, moisture, abundance of ammonium ions, population and diversity of nitrifying organisms, and the availability of substrate to

nitrifiers are most important (Focht and Verstraete 1977; Allen et al. 2005; Yuan et al. 2005). Light was identified as a major factor inhibiting nitrification in a wastewater reservoir in Israel. Under lack of light, nitrite oxidation was especially hindered, causing the accumulation of nitrite during late spring and summer (Kaplan, Wilhelm, and Abeliovich 2000).

At times, it is difficult to sort out the influence of individual environmental factors on nitrification, except in experiments under controlled conditions. For example, in the soil system, soil moisture and aeration or soil oxygen levels are inversely related. Oxygen content in the soil is reduced at higher soil moisture as most of pore spaces are occupied by water, and higher soil moisture also restricts diffusion of atmospheric air into the soil. Thus, optimum conditions for both moisture and aeration is critical for nitrification to take place in the soil.

The nitrobacteria are obligate autotrophs and produce nitrate in the presence of molecular oxygen, and nitrification takes place in well-aerated soils. Maximum rate of nitrification is achieved when oxygen is about 20% of the air and this concentration is similar to the oxygen concentration in the atmosphere (Black 1957; Tisdale and Nelson 1970).

Keeney, Sahrawat, and Adams et al. (1985) conducted experiments to study the effects of a range of carbon dioxide concentrations (ambient to 100%) on nitrification, denitrification, and associated nitrous oxide production in a silt loam. An increase in carbon dioxide concentration from 0.3 to 100% increasingly retarded the rate of nitrification in the soil. No nitrification occurred at 100% carbon dioxide. Nitrous oxide associated with nitrification increased as carbon dioxide increased from 0 to 2.6% and tended to be greater as carbon dioxide concentration increased to 73%. At 100% carbon dioxide, no nitrous oxide (no nitrification) was produced during 7 days at 25°C (Keeney, Sahrawat, and Adams 1985).

In a laboratory assay on the nitrification of <sup>15</sup>N-labeled ammonium sulfate in soil, the removal of formed carbon dioxide from the closed incubating vessel, by trapping in an alkali solution, decreased nitrification of ammonium sulfate. Nitrification was sensitive to available carbon dioxide, decreasing significantly when carbon dioxide was trapped in alkali solution and increasing substantially when the amount of carbon dioxide in the soil atmosphere increased as a result of decomposition of added wheat straw. The results suggest that decreased carbon dioxide concentration, resulting from its removal, in incubated soils, would retard nitrification rate in incubated soil (Azam et al. 2004).

Nitrification rate in soil is the maximum near-field capacity moisture (−33 kPa in medium to heavy-textured soils, and 0 to −10 kPa in light-textured or sandy soils). Nitrification in soils at a water potential of 0 kPa (saturated with water) is at low ebb or stops because of lack of molecular oxygen. Also, nitrification halts in dry soils, but it would appear that higher moisture content has greater adverse effect on nitrification than lower moisture content in the soil. It is reported that nitrobacteria may function

well in reasonably dry soils (Justice and Smith 1962), whereas higher moisture content drastically curtails nitrification (Parker and Larson 1962).

Nitrification has long been known to generally follow a bell-shaped temperature response curve with an optimum at 30–35°C. The effect of temperature on nitrification is climate dependent (Mahendrappa, Smith, and Christianson 1966). For example, Sabey et al. (1956), who studied nitrification of ammonium sulfate in three Iowa soils at temperatures ranging from 8 to 30°C, found that nitrification reached a maximum at about 25°C. These results are in agreement with those by Justice and Smith (1962), who reported that nitrification in soil proceeded more rapidly at 25°C than at 35°C.

Myers (1975), on the other hand, who studied nitrification in a tropical soil (Tindall clay loam) from Australia over a temperature range of 20–60°C, found that the optimum temperature for nitrification was close to 35°C. The potential nitrification rate at 35°C was 4.8 mg N kg<sup>-1</sup> soil day<sup>-1</sup>, compared with 0.5 mg kg<sup>-1</sup> day<sup>-1</sup> at 20°C and 0.25 mg N kg<sup>-1</sup> day<sup>-1</sup> at 60°C.

These results demonstrate that the relationships describing temperature and nitrification may vary from one climate to another. Also, tropical soils appear to have a higher optimum temperature range compared to soils in the temperate regions for nitrification. Interestingly, the optimum temperature for nitrification in pure culture of nitrifiers has also been reported to be in the range of 25–35°C (Focht and Verstraete 1977).

At the ecosystem level, several environmental factors act together to influence nitrification and N cycling. For example, studies made in forest soils suggested that the nitrifier populations were higher in soil under successional than in more mature forests (Todd et al. 1975). Consequently, the rates of nitrification were higher in soils under early successional than those under more mature forest (Montagnini et al. 1986). It has also been reported that clear-cutting of coniferous forest influences differently the ammonia-oxidizing community in limed and unlimed soils. Clear-cutting caused a shift in the ammonia-oxidizer community, and the prior liming of the soil increased the responsiveness of the ammonia-oxidizers to the changes caused by cutting (Bäckman et al. 2004).

Sahrawat, Keeney, and Adams (1985) studied the rate of aerobic nitrogen transformations in six acid climax forest soils. They found that nitrate formation did not take place in the soils under natural pH (<5.0); application of calcium carbonate increased the pH and initiated nitrate formation in all the soils. The addition of phosphorus (P), however, had no effect on nitrification in soils. The results of this study do not support the hypothesis that the deficiency of nutrients such as P affects nitrification and nitrate production in acid climax forest soils.

Carney, Matson, and Bohannon (2004) found that in a tropical system in Costa Rica, the population of ammonia-oxidizing bacteria, diversity of composition, and nitrification rates did not change significantly across plant diversity treatment. However, the ammonia-oxidizing bacteria population and composition differed among land-use types, and the differences in ammonia-

oxidizing bacteria composition among land-use types were correlated with potential rates of nitrification in the soil. Nitrification potential ( $\text{mg nitrate-N kg}^{-1} \text{ soil h}^{-1}$ ) was highest in the soil under forest (1.36), followed by plantation and pasture (0.19). Soil properties such as extractable P, soil moisture, and C–N ratio did not significantly differ in soils under various land uses, although carbon (%) and microbial biomass significantly differed among the land-use systems (Carney, Matson, and Bohannan 2004).

The differential influence on nitrification and net nitrate production at the ecosystem level is ascribed to several factors, including population of nitrifying organism, stage of succession, plant species, nutrient status, and the presence of allelopathic chemicals in the soil system, although the mechanisms involved are not fully understood (Meiklejohn, 1968; Purchase 1974; Lodhi 1978; Donaldson and Henderson 1990; Stienstra, Klein Gunnewiek, and Laanbroek 1994; Knops, Bradley, and Wedin 2002; Lata et al. 2004).

However, it must be stated that the involvement of allelopathic chemicals on the nitrification process is far from fully established and at times controversial (Rice and Panchoy 1972, 1973, 1974; Vitousek et al. 1982; White 1994; Ste-Marie and Pare, 1999; Jafari and Kholdebarin 2002; Subbarao et al. 2006). This is due to complex interactions among the various factors on nitrification and net nitrate production. Additionally, the production of nitrate in forest soils is also affected by former cultivation, fertilization and manuring practices, and the past history of management, which need to be considered for nitrate production and its movement in forest ecosystem soils (Glatzel 1991; Stark and Hart 1997; Jussy et al. 2002).

In laboratory experiments, Bremner and McCarty (1988) found that the application of terpenes to agricultural soils as vapors or directly even at high concentrations had no significant effect on nitrification. The authors, however, reported that organic carbon in the form of terpenoids was as effective as glucose at stimulating microbial immobilization of ammonium-N. White (1991), on the other hand, reported that the application of pine monoterpenes inhibited N mineralization and nitrate formation when added at low concentration. However, the application of terpenes at higher concentration resulted in the immobilization of ammonium-N.

These results suggest that lack of nitrate production or its low concentration in soil could also be caused by immobilization and unavailability of ammonium for nitrification rather by the retardation of nitrification (Sahrawat 1989, 1996). Thus, it is of critical importance to ascertain whether the lack of nitrate production is due to retardation of nitrification or simply the result of immobilization of ammonium. Because both retardation of nitrification and immobilization of ammonium could lead to same result, especially if the chemicals involved are natural products and they are added at higher concentration (Sahrawat 1988).

Nevertheless, there is evidence to show that in forest ecosystems the availability of ammonium is the main factor regulating nitrification (Montagnini, Haines, and Swank 1989, Ste-Marie and Pare 1999). Thus the

allelopathic effects of chemicals on nitrification under succession in forest and grassland ecosystems remain controversial and far from fully established (Subbarao et al. 2006).

Upadhaya et al. (2005) studied N mineralization and nitrification in relation to the dynamics of fine and coarse roots in a humid subtropical forest ecosystem of northeast India. They found that the total, fine, and coarse root mass in the protected stands (undisturbed) were higher than those recorded in the unprotected stands (disturbed by human activity). The mean concentration of ammonium-N and nitrate-N was markedly higher in the protected stands than in the unprotected stands. The N mineralization and nitrification rates were significantly higher in the soil surface layer (0–10 cm) than in the subsurface soil (10–20 cm). The results showed that disturbance in the forest caused a reduction in fine root mass as well as in N mineralization and nitrification (Upadhaya et al. 2005).

### Chemical Factors

Nitrification in a soil is affected by the availability of ammonium ions to the populations of nitrifying organisms, which in turn is influenced by the quality of soil organic matter, especially the C–N ratio. High C–N ratio leads to the immobilization of ammonium (Tisdale and Nelson 1970; Focht and Verstraete 1977; Sahrawat 1996).

Soil pH is the major factor regulating the nitrification process in soils. Nitrification takes place in soil at pH ranging between 5.5 to about 10.0, with the optimum around 8.5. However, nitrification has been reported to occur in soils with pH as low as 3.8 (Tisdale and Nelson 1970). Heterotrophs may contribute to nitrification in acid soils, but it is difficult to demonstrate their involvement conclusively (De Boer and Kowalchuk 2001).

Sahrawat (1982) studied nitrification in 10 soils (8 mineral and 2 Histosols) having a range in texture, pH (3.4 to 8.6), organic C (12.2 to 227.0 g kg<sup>-1</sup>), and total N (900 to 12000 mg kg<sup>-1</sup>). The amounts of nitrate-N produced at 30°C after 4 weeks of incubation of the soils varied from 0 to 123 mg kg<sup>-1</sup> soil. Soils with pH less than 5.0 did not nitrify at all; the organic soil with pH 5.6 produced only 5 mg nitrate-N kg<sup>-1</sup> soil during the period. Soils having pH of more than 6.0 nitrified at a rapid rate and released nitrate-N ranging from 98 to 123 mg kg<sup>-1</sup> soil (Table 1). The amounts of nitrate-N produced in the soils were highly positively correlated with soil pH ( $r = 0.86$ ,  $n = 10$ ,  $P < 0.01$ ) but not significantly correlated to organic C or total N. Statistical analysis of the data also showed that nitrate formation was not significantly correlated with soil pH in soils having pH higher than 6.0 ( $r = 0.13$  not significant,  $n = 6$ ) (Sahrawat 1982).

A study of nitrification in six acid climax forest soils from Wisconsin (USA) showed that nitrification did not occur at natural soil pH (<5.0), but liming of the soils increased pH and initiated nitrification in all soils



**Table 1.** Nitrification of soil nitrogen in 10 soils with a range in texture, pH, organic C, and total N, incubated aerobically at 30°C for 4 weeks (adapted from Sahrawat 1982)

Soil	pH (1:1 water)	Organic C (g kg <sup>-1</sup> )	Total N (mg kg <sup>-1</sup> )	NO <sub>3</sub> -N (mg kg <sup>-1</sup> )
Calalahan sandy loam	3.4	15.7	1100	0
Malinao loamy sand	3.7	12.2	900	0
Luisiana clay	4.4	15.2	1750	0
Morong peat	5.6 <sup>a</sup>	128.0	5600	5
Lam Aw peat	6.1 <sup>a</sup>	227.0	12000	116
Maahas clay	6.5	15.0	1200	106
Quingua silty loam	6.5	12.8	1150	115
Pila clay	7.5	22.7	1850	123
Lipa loam	7.5	25.0	1900	98
Maahas clay, alkalized <sup>b</sup>	8.6	15.0	1200	118

<sup>a</sup>pH measured on soil suspensions using a soil-to-water ratio of 1:2.

<sup>b</sup>Maahas clay plus 1.3% Na<sub>2</sub>CO<sub>3</sub>.

(Sahrawat, Keeney, and Adams 1985). In another study, Sahrawat (1980) studied N mineralization and nitrification in two acid sulfate soils (pH 3.4 and 3.7) incubated for 2 weeks at 30°C under aerobic conditions. Nitrification did not take place in either of the soils under conditions that stimulate nitrification. Mineralized N accumulated entirely as ammonium-N.

Kyveryga et al. (2004) conducted field studies for 4 years to assess the importance of soil pH on nitrification of the fall-applied anhydrous ammonia in the corn-belt region of the United States. Means of the measurements made on nitrate formation in mid-April, at the time of commencement of planting, indicated 89% nitrification of fertilizer N in soils having pH greater than 7.5 and 39% nitrification of fertilizer N in soils having pH less than 6.0. Significant relationships between soil pH and percentage nitrification of fertilizer N were observed each year.

The results discussed support the general conclusion that nitrification is severely curtailed at soil pH less than 5.0 and that nitrification is rapid in soils with pH greater than 6.0.

## CONCLUSIONS

The various factors that influence nitrification in soils are discussed with examples from natural and agro-ecosystems. Clearly, several physical, environmental, and chemical factors and interactions among them influence soil nitrification in a complex manner. However, among the various factors, soil matrix, moisture status, aeration, pH, and temperature play predominant roles in the nitrification of soil or added ammonium to nitrate. The information



on factors that influence nitrification is helpful for developing chemical and biological nitrification inhibitors to retard nitrification in soils. A more systematic approach is needed in this area of research (Sahrawat and Keeney 1985; Sahrawat 2003).

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