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Scope and Strategies for Regulation of Nitrification in Agricultural Systems—Challenges and Opportunities

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Nitrification, a microbial process, is a key component and integral part of the nitrogen (N) cycle. Soil N is in a constant state of flux, moving and changing chemical forms. During nitrification, a relatively immobile N-form (NH_4^+) is converted into highly mobile nitrate-N (NO_3^-). The nitrate formed is susceptible to losses via leaching and conversion to gaseous forms via denitrification. Often less than 30% of the applied N fertilizer is recovered in intensive agricultural systems, largely due to losses associated with and following nitrification. Nitrogen-use efficiency (NUE) is defined as the biomass produced per unit of assimilated N and is a conservative function in most biological systems. A better alternative is to define NUE as the dry matter produced per unit N applied and strive for improvements in agronomic yields through N recovery. Suppressing nitrification along with its associated N losses is potentially a key part in any strategy to improve N recovery and agronomic NUE. In many mature N-limited ecosystems, nitrification is reduced to a relatively minor flux. In such systems there is a high degree of internal N cycling with minimal loss of N. In contrast, in most high-production agricultural systems nitrification is a major process in N cycling with the resulting N losses and inefficiencies. This review presents the current state of knowledge on nitrification and associated N losses, and discusses strategies for controlling nitrification in agricultural systems. Limitations of the currently available nitrification inhibitors are highlighted. The concept of biological nitrification inhibition (BNI) is proposed for controlling nitrification in agricultural systems utilizing traits found in natural ecosystems. It is emphasized that suppression of nitrification in agricultural systems is a critical step required for improving agronomic NUE and maintaining environmental quality.

Keywords agriculture, biological nitrification inhibition (BNI), environment, global warming, nitrogen-use efficiency, nitrification inhibitors

I. INTRODUCTION

Human activities have profoundly influenced the global nitrogen (N) cycle. Nearly 234,650 Mg (metric ton) of gaseous atmospheric N covers each hectare of the Earth's surface; however, only plants in a symbiotic relationship with bacteria or cyanobacteria are able to utilize this vast reservoir of gaseous N (Mulder *et al.*, 1969). Most food crops such as cereals do not have the necessary symbiotic relationship and hence must rely on the fixed N that is derived from soil organic matter (SOM) mineralization, or supplied by N fertilizers. Most modern high-production cropping systems depend primarily on meeting the crop N needs with N fertilizer inputs as the N derived from SOM is generally inadequate for achieving high yields (Evenson and Gollin, 2003). Anthropogenic input of ammonia into the N cycle (i.e., obtained primarily through industrially fixed N fertilizer and cultivation of nitrogen-fixing crops such as legumes) currently exceeds contributions from natural sources of fixed N (Vitousek *et al.*, 1997).

The current fertilization philosophy in high-production agriculture seems to be to make certain that fertilization is never a limiting factor; this has resulted in the application of an excess of fertilizer (especially N) with little concern for the effect on

the environment. Massive amounts of fixed-N are used to drive the current intensive agricultural production systems to feed the global population. These large N inputs currently estimated at 100 Tg (million Mg) from industrially fixed-N (mostly in the form of ammonia) have many unintended environmental consequences, largely due to nitrification and associated N losses from agricultural systems. It is necessary, therefore, to develop strategies for the efficient use of N in crop production that would meet the high production requirements and also address the growing environmental concerns.

The biological oxidation of ammonia to nitrate is termed "nitrification" (Jarvis, 1996) (Figure 1). The nitrification section offers the most potential (in the N cycle) for changes that could increase nitrogen-use efficiency (NUE). Just by changing the form of N to nitrate makes it very susceptible to being denitrified or leached from the root zone. This change of form also affects on how N is absorbed by plants and the form of N that enters the N cycle or is lost to the environment (Norton *et al.*, 2002) (Figure 1).

A. Nitrification—Role in N Cycling

Nitrification along with N fixation and denitrification are the key processes that have a profound influence on the terrestrial N cycle (Figure 1). Nitrification is carried out by two groups of chemo-lithotropic bacteria (*Nitrosomonas* sp. and *Nitrobacter* sp.), both of which are ubiquitous components of all soil microbial populations. Nitrifiers have slow turnover rates, and possess the ability to survive long periods in a state of dormancy (Belser, 1979). Other soil bacterial spp. such as *Nitrosocystus* and *Nitrosospira*, and some heterotrophic fungi such as *Aspergillus flavus* also play a significant role in nitrification in some ecosystems (Sommer *et al.*, 1976).

1. Is Nitrification Causing Low NUE in Agricultural Systems?

The rapid conversion of NH_4^+ to NO_3^- in the soil limits the effectiveness of much of the applied N fertilizer. Nearly 90% of all the added N fertilizer is applied in the NH_4^+ form, which is mostly nitrified within four weeks after application (Sahrawat, 1980a). For most arable soils, nitrification is so universal and rapid that applications of NH_4^+ -N can generally be considered as almost being the equivalent of the application of NO_3^- -N (Mason, 1992; Strong and Cooper, 1992). Being a cation, ammonium (NH_4^+) is held by electrostatic forces to negatively charged clay surfaces and functional groups of SOM. This binding is sufficient to limit N losses by leaching (Amberger, 1993). Thus, nitrification of NH_4^+ -N results in the transformation of N from a relatively immobile N-form (i.e., NH_4^+) to a highly mobile N-form (NO_3^-), providing a much greater potential for N to be leached beyond the rooting zone. This transformation also provides many opportunities for N to escape into the environment as gaseous molecules (N_2O , NO , and N_2) (Figure 1). These losses of N lower the effectiveness of N fertilization and can have serious environmental implications when excess N

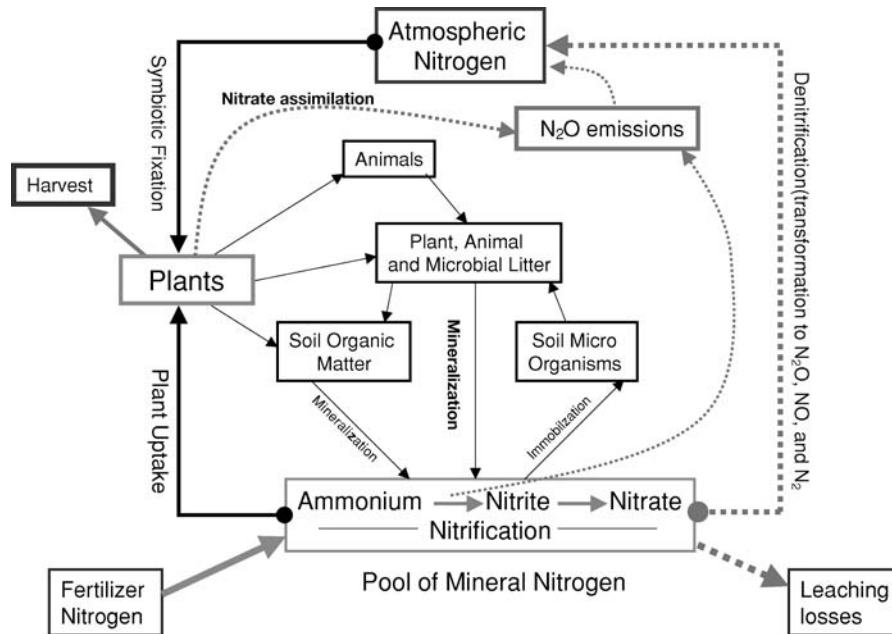


FIG. 1. Nitrogen cycle in soil.

enters the natural environment (Jarvis, 1996). Except for the volatilization of ammonia (Grant *et al.*, 1996), nitrification is associated with most of the major pathways of N loss, denitrification and NO_3^- leaching (Barker and Mills, 1980). From an agricultural viewpoint, maintaining N in the NH_4^+ form has the advantage of extending the time N remains in the rooting zone, providing more time for plant absorption (Slangen and Kerkhoff, 1984).

2. Are Systems Moving Towards High Rates of Nitrification?

Nitrification seems to play a relatively minor role in many climax communities of natural ecosystems which is in contrast to high-production agricultural systems, where nitrification is a major process impacting N cycling (Haynes and Goh, 1978). Modern agricultural systems heavily depend on large inputs of N fertilizer to maintain productivity, as naturally fixed N is seldom adequate for high-production systems (Dinnes *et al.*, 2002; Evenson and Gollin, 2003). Legumes, using their ability to symbiotically fix atmospheric N with rhizobia, supplement the amount of natively fixed N available to crops. During the 20th century, several significant changes have occurred as modern high-production systems developed (Rabalais *et al.*, 1996):

1. less use of diversified crop rotations
2. separation of crop production systems from animal enterprises
3. changes in soil tillage intensity
4. more irrigation and drainage of agricultural fields
5. increased use of N fertilizer

During the 1950s, cereals were commonly grown in rotation with legumes (such as soybean, chickpea, pigeonpea, cowpea, common bean, peas, alfalfa, red clover, sweet clover). Symbiotically fixed N plus N from mineralized SOM and animal manure were the primary sources of N for most cereal production both in the developed and developing regions of the world (Dinnes *et al.*, 2002). Following World War II, an increase in the availability of inexpensive N fertilizer plus a decreased demand for forage crops led to significant reductions in the use of crop rotations. In general, N fertilizer has replaced crop rotations as a principal source of N in many parts of the world. With the increased use of N fertilizer, animal manure is no longer used as a major crop nutrient resource. This encouraged the separation of crop and animal production enterprises which makes the recycling of animal wastes more difficult and less economical. Another unintended side effect of the large-scale use of N fertilizer is a general worldwide reduction of SOM (McGill *et al.*, 1981; Lal, 2003; Bellamy *et al.*, 2005). In addition, the installation of sub-surface drainage systems in many developed parts of the world, has led to acceleration in NO_3^- leaching, resulting in a substantial reduction in N-cycling efficiency (i.e., NUE) (Dinnes *et al.*, 2002). Modern high-production agricultural systems result in conditions that enhance nitrification, lower NUE and reduce SOM (McGill *et al.*, 1981; Peng *et al.*, 2005).

3. The Positive Impacts of Nitrification on N Cycling

Nitrification in some cases can lead to N retention, especially in alkaline soils where N losses from ammonia volatilization can be high. Under conditions of high pH, it is possible for nitrification to facilitate N retention by rapidly converting ammonia to

nitrate-N, which is not liable to loss by volatilization (Sahrawat, 1989). Nitrification followed by denitrification also plays a positive role in the management and recycling of organic wastes including nitrogenous wastes from animal and human excreta, sewage sludge and N originating from pulp, paper and other industries. Ammonia is the predominant form of N in these wastes, and nitrification is the starting process for N removal from these wastes before they are released into the environment (Kowalchuk and Stephen, 2001).

B. Soil Organic Matter (SOM) as a Source of NH_4^+ for Nitrification

There is a general misconception that nitrification-associated problems, NO_3^- leaching, N_2O and NO emissions, are exclusively associated with inorganic N fertilizer applied to agricultural systems. Native soil organic matter (SOM) also goes through mineralization (proteolysis and ammonification) resulting in the release of substantial amounts of NH_4^+ -N to the soil (Mengel and Kirkby, 1978; Addiscott, 2000; Chikowo *et al.*, 2004). Irrespective of its origin, either from N fertilizer or SOM derived N, NH_4^+ goes through the same nitrification and is also lost due to similar processes. In many agricultural systems, SOM-derived N (i.e., mineralized N) contributes substantially to NO_3^- leaching losses when the land is left fallow. Nitrification proceeds rapidly for N derived from SOM during the early summer after the crop is harvested, and continues throughout the summer. The NO_3^- that is formed from SOM is often leached out of the rooting zone during early spring rains (Russell, 1914). For many arable soils, nearly $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ of mineralized N is lost through the leaching of NO_3^- (Russell, 1914).

On a global scale, soil organic N is the dominant pool of N in terrestrial ecosystems, being several times higher than the pool of N in plant biomass (Powlson, 1993). The surface layer of arable soil (top 30 cm) typically contains 2 to 6 Mg N ha^{-1} in organic matter (OM). This is based on the assumption that $4.5 \times 10^6 \text{ kg}$ of soil ha^{-1} are in the surface 30 cm, with an average C/N ratio of 10:1 for the SOM; this translates into 4.5 Mg N ha^{-1} for every 1% SOM, with the most fertile arable soils containing about 3% OM. Out of this total organic N, about $3\% \text{ yr}^{-1}$ is mineralized, and is initially available in the NH_4^+ form, but proceeds rapidly through nitrification to NO_3^- at a rate of about 45 to $140 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Bremner, 1965; Powlson, 1993).

Nitrogen from SOM can be a significant part of the N pool in agricultural soil subject to loss through leaching and gaseous emissions (i.e., N_2O and NO) (Bremner, 1965; Whitmore *et al.*, 1992). When making decisions about N fertilizer applications, farmers rarely take into account the N derived from SOM, and this often results in overfertilization with the excess N being lost to the environment (Dinnes *et al.*, 2002). A study using soils with high levels of SOM (close to 10%) showed that nearly $70 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ leached down the soil profile (Rennie *et al.*, 1976). Because of nitrification-associated N losses in organic soils, even soils with large amounts of mineralizable SOM ($>200 \text{ kg N ha}^{-1}$

yr^{-1}) may be limited in their N supplying capacity and not be able to meet the N demands of a rapidly growing crop (Guthrie and Duxbury, 1978).

C. Crop Preference for Nitrogen Form

Most crops have the ability to take up and utilize both NH_4^+ and NO_3^- (Haynes and Goh, 1978). Usually a mixture of NH_4^+ and NO_3^- is preferable as the N source (Michael *et al.*, 1970). However, many dry-land crops show a preference for NO_3^- over NH_4^+ (Haynes and Goh, 1978). Crops that are well adapted to anaerobic soil conditions (such as rice) and plants that are adapted to acidic soils generally have a preference for NH_4^+ over NO_3^- (McKane *et al.*, 2002). For detailed discussions on NH_4^+ as N source for plants, readers are referred to Haynes and Goh (1978).

D. Theoretical Basis for Nitrification as a Selective Pressure in Biological Systems

Assimilation of NO_3^- requires an energy equivalent to 20 moles of ATP mole^{-1} of NO_3^- , whereas NH_4^+ assimilation requires only 5 moles of ATP mole^{-1} of NH_4^+ (Salsac *et al.*, 1987). This difference in energy requirement for the assimilation of different forms of N has been proposed as a possible ecological driving force in the development of climax ecosystems. Inhibition of nitrification is a possible ecological mechanism that could take advantage of the difference in energy efficiency in the assimilation of NH_4^+ and NO_3^- (Rice and Pancholy, 1972). Energy savings from the assimilation of NH_4^+ should lead to higher production of biomass from plants grown with NH_4^+ -N over NO_3^- -N. However, for most agricultural systems, a balance of two thirds to one third between NO_3^- and NH_4^+ often provides the best crop growth and productivity.

E. Scope and Outline of This Review

In recent decades, human activity has greatly increased the availability of fixed N in both natural and agro-ecosystems. Nitrification of this fixed N opens up a number of pathways to N losses resulting in a lower agronomic NUE. In addition, there are environmental costs associated with these N losses such as greenhouse gas emissions and the pollution of streams and lakes. Nitrogen cycling in high-input agricultural systems is substantially less efficient than in most mature ecosystems where the N cycle is fairly closed. For the overall efficiency and ecological sustainability of high-production agricultural systems, N cycling needs to be made as efficient as possible. It appears that much of the efficiency found in natural systems is associated with plant utilization of N directly from the NH_4^+ form before nitrification occurs. It would be desirable if we could introduce/transfer these mechanisms into agricultural systems using strategies that combine management and genetic modification. This review aims to present the current state of knowledge and perception of the impact of nitrification on N recovery and utilization by natural and agricultural ecosystems. We also discuss strategies and

possible mechanisms for controlling nitrification in agricultural and natural ecological systems.

II. NITRIFICATION—ITS ENVIRONMENTAL IMPACT

Nearly 70% of the applied N fertilizer from managed ecosystems is lost through nitrification and associated processes (Raun and Johnson, 1999; Glass, 2003). Also, nitrification-associated losses (NO_3^- leaching and denitrification) from mineralized SOM and organic residues can be substantial, largely due to lack of synchronization between organic N mineralization and crop N utilization (Addiscott, 2000; Chikowo *et al.*, 2004). Nitrogen fertilizer accounts for a significant amount of the total energy input into agricultural systems. Nitrogen incorporated into agricultural crops rarely exceeds 40% of the applied N with an average of about 32%, indicating a serious inefficiency in N and energy utilization in current high-production agricultural systems (Raun and Johnson, 1999; Glass, 2003). Recent research shows that N is also lost as N_2O emissions by plants during the process of NO_3^- assimilation in leaves (Smart and Bloom, 2001).

A. Recovery of Fertilizer N in Agricultural Systems

One of the main causes for the poor N recovery is nitrification, which is rapid in arable soils (Sahrawat, 1980a). The nitrate formed in this process is subject to substantial losses through multiple mechanisms and pathways (Figure 1). The green revolution led to nearly doubling/trebling the grain yields of major food crops (corn, wheat, and rice), but at the same time decreased agronomic NUE (Bock and Hergert, 1991; Peng *et al.*, 2005). Recovery of N fertilizer varies among crops. For many tropical production systems, N recoveries range between 30 to 40% (see review by Prasad and Power, 1995), and in some extreme cases recoveries of N as low as <10% are reported for rice (Peng *et al.*, 2005). For high-N-input (300 to 900 kg N ha⁻¹ yr⁻¹) plantation crops such as coffee and green tea, <30% of the N is recovered (Babbar and Zak, 1995). For pasture grasses, N recoveries are close to 60%, which is generally higher than those for grain crops (Prasad and Power, 1995). In many fertile soils, mineralizable SOM can substantially contribute to the available pool of N, but is often not taken into account when calculating N fertilizer requirements. This results in the application of excess N and its subsequent inefficient use (Dinnes *et al.*, 2002).

B. Losses of N Associated with Nitrification

The two major pathways of N loss during and following nitrification are gaseous emissions as dinitrogen (N_2) and oxides of N (N_2O , NO), and the leaching of NO_3^- .

1. Gaseous Nitrogen Losses (N_2O , NO, and N_2)

Nitrogen is lost in gaseous forms from agricultural systems:

1. During biological oxidation of NH_4^+ -N to NO_2 -N by *Nitrosomonas* [i.e., during hydrolysis of hydroxylamine by

hydroxylamine oxidoreductase (HAO) enzyme], N_2O is emitted (Bremner and Blackmer, 1978).

2. Under anaerobic conditions, denitrification of nitrate (i.e., conversion of nitrate into gaseous forms— N_2O , NO, N_2) by heterotrophic soil bacteria (*Bacillus subtilis*, *E. coli*, *Achromobacter aerogenes*, *Aspergillus flavus*, *Pseudomonas* sp., *Micrococcus* sp., and *Penicillium atrovirens*) (see Mosier *et al.*, 1996).
3. During nitrate assimilation by plants, N_2O is emitted (Smart and Bloom, 2001).

a. Gaseous Nitrogen Losses (N_2O , NO and N_2) Associated with Nitrification and Denitrification. Nitrous oxides are produced and emitted from soils during nitrification under aerobic conditions (up to 50% of the soil pore space filled with water) and denitrification of NO_3^- -N under anaerobic conditions (>75% of the soil pore space filled with water) (Mosier *et al.*, 1996). These two processes contribute nearly 90% of the N_2O emissions from agricultural systems (Smith *et al.*, 1997). Potentially both N_2O and NO may evolve during nitrification in soils; usually NO emission is 10 to 100 times larger than that of N_2O (Hutchinson and Davidson, 1992). N_2O emissions become relatively more important with increases in soil moisture (Hutchinson and Davidson, 1992). Nitrogen lost as N_2O during nitrification rarely exceeds 2% of the total N nitrified, but sometimes the losses may reach up to 4% (Duxbury and McConnaughey, 1986) making this pathway of limited significance for total N lost (Bremner and Blackmer, 1978; Mosier *et al.*, 1996). In some extreme cases such as in drained organic soils, where mineralization of organic-N could reach up to 1400 kg N ha⁻¹ yr⁻¹, N losses as N_2O emissions could reach as high as 100 kg N ha⁻¹ yr⁻¹ (Terry *et al.*, 1981).

The N losses as N_2O from denitrification however, are substantial (Davidson, 1991) and can occur very rapidly (within 24 h) in soils that are wet (but not water-logged; about 70% of the soil pore space water-filled) (Linn and Doran, 1984) and sufficiently warm to support rapid microbial activity (Sahrawat and Keeney, 1986). Nearly 60% to 80% of the NO_3^- -N can be lost in gaseous forms (N_2O , NO and N_2) under anaerobic conditions (Mosier *et al.*, 1996). In rice production systems where alternate drying and wetting is a common feature of the agronomic practice, denitrification is a major pathway of N loss (De Datta *et al.*, 1991). Losses of N by denitrification are also influenced by SOM levels, which indirectly determine the degree of heterotrophic bacterial activity involved in denitrification (Barker and Mills, 1980). Agronomic practices, and in particular SOM management can influence N losses through their effects on denitrification. For example incorporation of wheat straw into the soil increases N loss by denitrification (Aulakh *et al.*, 1984). Similarly, leaving legume residues such as soybean on the soil surface enhances denitrification (Mosier, 1994). Also, denitrification losses from NO_3^- fertilizers and organic-N fertilizers such as animal waste are generally much higher than those from NH_4^+ -based or ammonium-forming fertilizers (Bremner *et al.*, 1981).

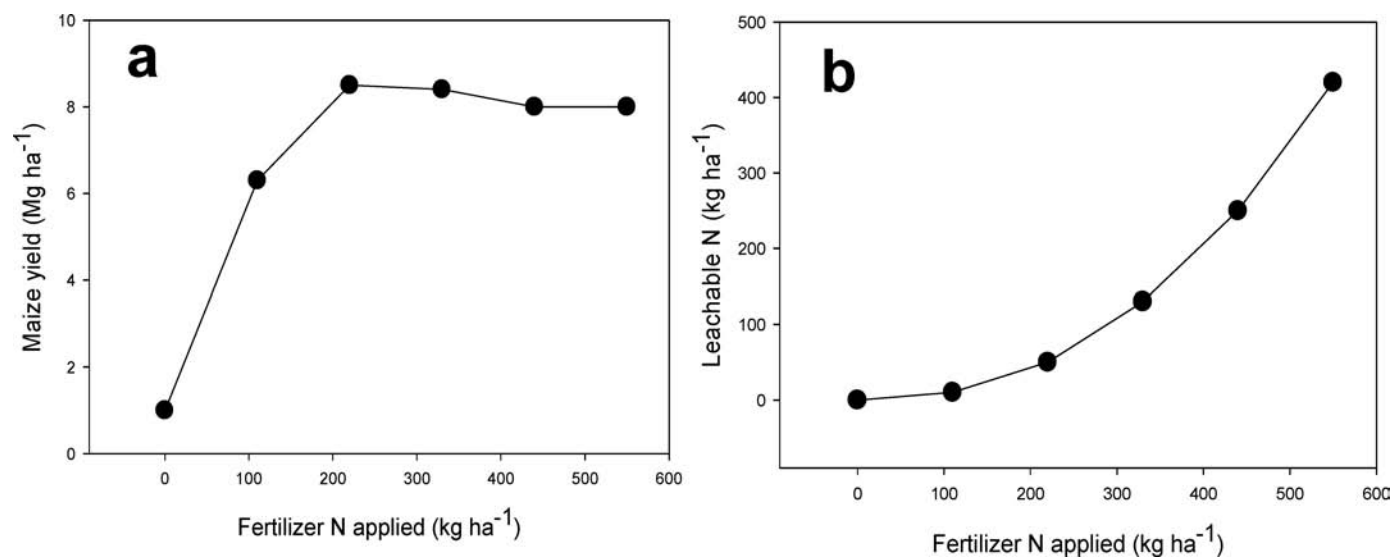


FIG. 2a, b. Leachable soil nitrogen as a function of fertilizer nitrogen applied and plant uptake in corn (adapted from Broadbent and Rauschkolb, 1977).

Plants may enhance the denitrifying activities of heterotrophic bacteria by supplying carbon through root exudation, thus accelerating N losses from NO_3^- -N (Cheng and Coleman, 1990; Wheatley *et al.*, 1990). Also, N_2O emissions differ among crops; field plots with legumes such as soybean, alfalfa, and clover may emit larger amounts of N_2O compared with cereal crops such as wheat (Smith *et al.*, 1997). In addition, rhizobia in root nodules can denitrify and produce N_2O (Mosier *et al.*, 1996).

b. Emissions of N_2O Associated with Assimilation of NO_3^- -N in Plants. Recently, it was shown that wheat plants emit N_2O during NO_3^- assimilation in their leaves (Smart and Bloom, 2001). Wheat emitted N_2O when grown on NO_3^- -N, but not when grown on NH_4^+ -N, and N_2O emissions were correlated with leaf NO_3^- assimilation activity (Smart and Bloom, 2001). Using ^{15}N isotopes, it was demonstrated that N_2O production was from the plant and not by microorganisms. The enzymes responsible for NO_3^- assimilation in plants are NO_3^- reductase (NR) and NO_2 reductase (NiR), located in the cytosol and chloroplasts, respectively. Based on the available evidence, about 0.2% of the NO_3^- -N assimilated by wheat can be lost as N_2O (Smart and Bloom, 2001). Terrestrial plants assimilate about 1200 Tg of N yr⁻¹ (Schlesinger, 1997). Nearly half of this N is absorbed and assimilated as NO_3^- (Raven *et al.*, 1993), of which 25–75% is assimilated in the leaves (Andrews, 1986). Thus if all terrestrial plants behaved the way wheat plants do, N_2O emissions due to NO_3^- -N assimilation would represent 5–6% of the total amount of N_2O -N emitted from agricultural systems (Schlesinger, 1997).

2. N losses by NO_3^- Leaching

NO_3^- readily moves through the soil by diffusion and with the mass flow of water (Vitousek *et al.*, 2002; Herrmann *et al.*, 2005); thus there is the potential for a significant portion of the

applied N fertilizer and naturally mineralized N to be leached out of the root zone (Baber and Wilson, 1972; Gulliam *et al.*, 1985). Several factors contribute to the leaching and runoff of NO_3^- -N that include soil type, climatic conditions, and field drainage characteristics (Burns, 1977; Dinnes *et al.*, 2002). Both tropical and temperate cropping systems offer potential for high NO_3^- leaching (Goss *et al.*, 1988; Schroder *et al.*, 1993). For example, based on long-term field experiments in England (about 140 years), leaching losses of NO_3^- -N up to 100 kg N ha⁻¹ yr⁻¹ were observed from silty clay loam soils (Johnston *et al.*, 1989). Coarse-textured soils are especially susceptible to leaching; nearly 60% of the applied N fertilizer can be lost to leaching in these soils (Gaines and Gaines, 1994). Also, NO_3^- leaching is influenced by the amount of excess N available (Scheppers *et al.*, 1991) (Figure 2). About 53% of the applied N fertilizer or nearly 80 kg NO_3^- -N ha⁻¹ yr⁻¹ leached from some citrus orchards in the Santa Ana Basin (USA) on sandy loam soils (Davis and Grass, 1966; Bingham *et al.*, 1971). Nitrate-N leaching from some of the highly fertilized and irrigated soils in this basin can be as high as 912 kg NO_3^- ha⁻¹ yr⁻¹ (Adriano *et al.*, 1972; Pratt and Adriano, 1973).

Grasslands can lose nearly 40% of the 400 kg N ha⁻¹ yr⁻¹ applied through NO_3^- leaching (Ryden *et al.*, 1984). Intensively managed pasture production systems of Europe and Australia lose substantial amounts of NO_3^- -N by leaching, reaching as high as 400 kg N ha⁻¹ yr⁻¹ (Garwood and Tyson, 1973; Ball *et al.*, 1979). Also, substantial amounts of NO_3^- (200 kg N ha⁻¹ yr⁻¹) leach from ryegrass-white clover pastures, where N inputs are principally from the legume component of the pasture, through symbiotic N fixation (Ball *et al.*, 1979; Hoglund *et al.*, 1979). In addition, SOM mineralization contributes to NO_3^- -N leaching in many agricultural systems (Addiscott, 2000; Chikowo *et al.*, 2004). Nearly 1.75 billion Mg of organic N is estimated to have been lost from U.S. agricultural soils

during the last century (Viets, 1975). Thus, irrespective of the source from which NO_3^- is derived, it is susceptible to loss by leaching.

Extensive studies carried out by the U.S. Geological Survey and the U.S. Environmental Protection Agency have shown that there is a strong correlation between intensive fertilizer use and NO_3^- concentrations found in well-waters in areas that are vulnerable to leaching (Hauck, 1990; Halvorson *et al.*, 2002). There is also evidence to show that the high concentrations of NO_3^- in shallow aquifers underlying major agricultural regions worldwide are related to agricultural activities, especially N-fertilizer practices (Schepers *et al.*, 1991; Davies and Sylvester-Bradley, 1995; Giles, 2005). Groundwater NO_3^- levels have risen up to 10-fold since the 1930s and have reached the limits set by WHO (50 mg NO_3^- or 11.3 mg NO_3^- -N L^{-1}) in many of the cereal-growing regions of England (Wilkinson and Green, 1982; Royal Society, 1983).

C. Environmental Impacts from Nitrogen Losses

The N losses associated with nitrification can be sufficiently large to have serious environmental and economic consequences to society (Mosier *et al.*, 1996; Smith *et al.*, 1997). Denitrification has a role in maintaining the composition of the atmosphere relative to oxygen and N levels. For millions of years, the rate of denitrification may have been balanced by N fixation (Crutzen and Ehhalt, 1977). Nitrous oxides that reach the stratosphere can destroy the protective ozone layer (Crutzen and Ehhalt, 1977; Crutzen, 1981). One of the more crucial features of the atmospheric ozone layer in the stratosphere is its ability to prevent the penetration of biologically damaging ultraviolet radiation to the surface (Crutzen and Ehhalt, 1977).

To sustain the current large human population requires the use of large amounts of industrially fixed N (Mosier *et al.*, 1996; Raun and Johnson, 1999). Nearly 100 Tg N yr^{-1} as fertilizer is currently applied worldwide to maintain agricultural production (Vitousek *et al.*, 1997; IFA, 2005). Nitrogen inputs from anthropogenic sources that include N fertilizer and N fixed biologically from leguminous crops now exceed N inputs from natural ecosystems (Vitousek *et al.*, 1997). Fertilizer N consumption will need to be doubled (i.e., to 200 Tg N yr^{-1}) by 2025 to meet the estimated increased demand for food (Crutzen and Ehhalt, 1977; Vitousek *et al.*, 1997). This increase in N use is also expected to increase both the nitrification and denitrification of N (Smith *et al.*, 1997). This type of N loss has been likened to a "hole in the pipe", where any increase in flow through the pipe (the main process) results in an increase in the absolute quantity escaping via the hole (the minor pathways) (Firestone and Davidson, 1989). The N loss that occurs during nitrification and denitrification is caused by inherent problems in the enzymatic conversion. The current estimations are that the N_2O emissions will double from the current 12.7 Tg N yr^{-1} to 25.7 Tg N yr^{-1} by 2025 (Kroeze, 1994). Doubling the concentration of N_2O in the atmosphere could result in a 10% decrease in the ozone

layer which would increase the ultraviolet radiation reaching the surface by 20% (Crutzen and Ehhalt, 1977).

1. NO and N_2O Emissions and Global Warming

In the atmosphere N_2O acts as a powerful greenhouse gas and absorbs infrared radiation coming from the Earth's surface that would otherwise escape back into the space. Absorption of infrared radiation limits the escape of energy from the Earth and traps it in the atmosphere which results in an increase in the average temperature of the planet, the so-called global warming phenomenon. A global increase of 0.6°C has been reported during the last century (IPCC, 2001). Although other trace gases in the atmosphere have the ability to trap infrared radiation, N_2O does it very effectively. The global warming potential (GWP) of N_2O is 296 times higher than that of CO_2 , and around 13 times higher than that of methane. This is the result of longer residence times in the atmosphere (132 years vs 10.5 years for methane) and a higher absorbance of infrared radiation (210 times higher than that of CO_2) (IPCC, 2001). Since the beginning of the industrial revolution in the 19th century, the concentration of N_2O in the atmosphere has increased from 270 ppb to the current 320 ppb (IPCC, 2001). N_2O concentrations in the atmosphere continue to rise at about 0.75% per year (Smith *et al.*, 1997; Stein and Yung, 2003). Agriculture is a major source of N_2O emissions, accounting for nearly 70% of the total anthropogenic emissions (Mosier, 1993; Smith *et al.*, 1997). The global emission of N_2O from cultivated land is now estimated to be about 3.5 Tg N_2O -N annually, of which 1.5 Tg is directly attributed to the use of synthetic N fertilizers (Kroeze, 1994; Smith *et al.*, 1997). Global emissions of N_2O from tropical forests are estimated at 2.2 to 3.7 Tg N_2O -N yr^{-1} , with a likely value of 3 Tg N, which is slightly lower than that emitted from agricultural lands (Prather *et al.*, 1995). By 2100, the total N_2O emissions originating from synthetic fertilizer production and use are projected to be 4.2 Tg N yr^{-1} , about 4 times that of current levels of emissions (Kroeze, 1994; Mosier *et al.*, 1996).

Although NO itself is not a greenhouse gas, it plays a key role in atmospheric chemistry. NO is a highly reactive molecule that easily combines with ozone to form NO_2 that, in turn, reacts with molecular O_2 to reform NO . This is an autofeedback catalytic cycle that destroys the ozone layer. NO can also be converted into nitric acid, which is recycled in the form of acid rain contributing to acidification and eutrophication of ecosystems. The emissions of NO associated with agriculture are about 30% of the total NO emitted from Earth, and this amounts to about 30 Tg NO -N yr^{-1} (Smith *et al.*, 1997). Most of these emissions are either directly or indirectly coupled with the process of nitrification, and are expected to increase proportionally with the anticipated increase in use of N fertilizer.

2. NO_3^- Pollution and Eutrophication

One of the problems associated with nitrification is the leaching of NO_3^- and its contamination of ground- and other

freshwater bodies (Scheepers *et al.*, 1991; Giles, 2005). Human and animal consumption of nitrate can lead to health risks that include methemoglobinemia in infants ("Blue Baby") (Shoval and Gruener, 1972) and NO_3^- poisoning in animals (NRC, 1972). Nitrate contamination of fresh-water systems often triggers eutrophication, by stimulating the growth of phototrophic and heterotrophic organisms that lead to an increase in their biomass and a decline in biodiversity. The excessive increase in biomass production creates anoxic conditions and a decrease in water quality (Vitousek *et al.*, 1997). Eutrophication of estuaries and other coastal marine environments is a major environmental concern worldwide due to its impact on fish and shellfish production. Nitrogen loadings through NO_3^- leaching of the Mississippi River and its tributaries were identified as the main cause for eutrophication of the Gulf of Mexico (Rabalais *et al.*, 1996; Dinnes *et al.*, 2002). The economic loss from the NO_3^- -N leached into the Mississippi River each year is estimated to be US\$ 750 million, which amounts to 15% of the total value of N fertilizer applied to all agricultural systems in the United States (Malakoff, 1998).

III. SOIL FACTORS INFLUENCING NITRIFICATION

Nitrification as a soil biological process is influenced by:

1. Soil physical factors including texture and structure
2. Soil environmental factors, which include temperature, moisture, aeration (i.e., O_2 and CO_2 levels)
3. Soil chemical factors such as pH, electrical conductivity (EC), C/N ratio, cation exchange capacity, and organic matter.

Plants themselves modify the soil physical, biological and chemical environment as they grow. They change carbon and nutrient availability through litter fall and exudates from roots and foliage. Nitrification is in a continuous state of flux in both managed (such as arable land) and natural ecosystems (forests and grasslands). The nitrification of NH_4^+ -N releases protons, which acidifies the soil. The natural buffering capacity of the soil influences the nitrification rate. Soils with a low buffering capacity will become acidified rapidly with the nitrification of NH_4^+ -N. Low soil pH reduces the rate of nitrification (De Boer and Kowalchuk, 2001).

A. Physical

Soil texture and structure influence N mineralization by affecting the aeration status, physical distribution of organic materials, and physical, chemical and biological environment in the soil (Strong *et al.*, 1999). The rate of nitrification is also a function of NH_4^+ absorption; the greater the absorption, the faster the rate of nitrification. Nitrifying bacteria grow on the surface of soil particles in close proximity to where the NH_4^+ -ions are held on the cation exchange complex (Harmsen and Van Schreven, 1955). Nitrifier growth rate is thus proportional to the surface

area on which NH_4^+ -ions are adsorbed, which is a function of cation-exchange capacity of the soil. The availability of NH_4^+ to nitrifiers in soil depends on ammonium fixation capacity of clay minerals, and the presence of other competing cations on the exchange sites, making nitrification in the soil a rather complex process (Sahrawat, 1979; Wang *et al.*, 2003).

B. Environmental

1. Aeration

In the soil environment, O_2 and CO_2 levels, temperature, and moisture are critical factors that affect nitrifier activity and nitrification (Focht and Verstraete, 1977; Yuan *et al.*, 2005). The interactions between moisture and O_2 status in the soil matrix have a major influence on nitrification. In the soil system, moisture and aeration are inversely related, thus it is difficult to differentiate the extent of their relative roles in soil nitrification. Oxygen concentration in the soil is reduced at higher soil moisture as most of the soil pore spaces are filled with water; in addition, high soil moisture also restricts diffusion of air into the soil (Focht and Verstraete, 1977). Nitrification is an aerobic process, which takes place in well aerated soils. Optimum nitrification rates are achieved when soil-oxygen levels reach about 20%, which is similar to O_2 levels in the atmosphere (Black, 1957; Tisdale and Nelson, 1970). Nitrifiers are autotrophs; thus they rely mostly on atmospheric CO_2 for their carbon requirements. Moderate levels of CO_2 in the range of 1 to 5% (by volume) stimulate nitrification in incubation vessels (Keeney *et al.*, 1985; Azam *et al.*, 2004). Nitrification is suppressed when soil incubation vessels are maintained below the normal atmospheric levels of CO_2 (Keeney *et al.*, 1985; Azam *et al.*, 2004).

2. Moisture Status

Nitrification rate in soil is near maximum when soil moisture is at field capacity (Myers *et al.*, 1982). In saturated soils, nitrification nearly stops due to lack of O_2 . Nitrification also stops in very dry soils (Justice and Smith, 1962; Parker and Larson, 1962).

3. Temperature

The optimum temperatures for nitrifiers in pure cultures range from 25 to 35°C (Focht and Verstraete, 1977). Nitrification in soils also shows a similar temperature optimum at 25 to 35°C (Justice and Smith, 1962; Mahendrappa *et al.*, 1966). The optimum temperature for the nitrifier activity in pure cultures or in soil is a function of the native environment of the nitrifiers. Soils from temperate climates and nitrifiers isolated from temperate regions have optimum temperatures for nitrification close to 25°C (Justice and Smith, 1962; Mahendrappa *et al.*, 1966), whereas the optimum temperatures for nitrifiers collected from tropical climates is around 35°C (Myers, 1975). Though nitrifier activity in soils is low at temperatures <15°C, some

nitrification still takes place in soil even at temperatures as low as 0°C (Focht and Verstraete, 1977).

C. Chemical Factors

1. pH

Optimum pH for the growth of nitrifiers in pure cultures is in the range of 6.7 to 8.5 (Kyveryga *et al.*, 2004), depending on the pH of the soil from which the nitrifiers were isolated (Quastel and Scholefield, 1951). Nitrification can occur in acid soils with a pH as low as 3.8, though at a much slower rate (Tisdale and Nelson, 1970). Generally nitrification is rapid in soils with a pH \geq 6.0, but slower in soils with a pH \leq 5.0. Under acidic conditions, ammonia occurs mostly as NH_4^+ , thus it is not suitable as a substrate for ammonia monooxygenase (Suzuki *et al.*, 1974). Nitrifiers isolated from acid soils fail to nitrify in liquid cultures at a pH below 5.5 (Prosser, 1989). Nevertheless nitrification proceeds in soils with a pH \leq 4.0 (Vitousek *et al.*, 1982). Several theories have been proposed to explain the nitrification in acidic soils including existence of acid-tolerant strains, microsites of relatively higher pH, syntrophic associations with mineralizing organisms, and protection via aggregate formation (Kowalchuk and Stephen, 2001).

Genetic adaptation of *Nitrosomonas* to acidic pH conditions is linked with the development of genetically distinct nitrifier populations in ecological niches with diverse pH habitats ranging from pH 3 to 10 (De Boer *et al.*, 1988). Molecular surveys (based on 16S rRNA gene sequence analysis) of ammonia-oxidizing bacteria (AOB) across pH gradients have identified *Nitrospora* sequence cluster-2 as putatively acidophilic AOB strains. *Nitrospora* sequence cluster-3 is mostly found in neutral pH soils (normal arable soils), whereas sequence cluster-2 mostly dominates in acidic soils (Stephen *et al.*, 1996). Using a vertical pH gradient in some acid forest soils, it was demonstrated further that sequence cluster-2 dominates in the upper-most organic layers of the soil that are mostly acidic (Kowalchuk and Stephen, 2001). Similarly, *Nitrospora* sequence cluster-2 dominates in several acidic grassland soils, and also in acidic Belgian forest soils where nitrification takes place at a near normal rate (Kowalchuk *et al.*, 2000). In contrast, *Nitrospora* sequence cluster-3 is the dominant nitrifying community in arable soils with a pH in the normal range (Kowalchuk *et al.*, 2000). Also, *Nitrospora* sequence cluster-3 appears adapted to high-N environments (i.e., arable lands where nitrogen fertilizers are applied at high frequency), and does not have the ability to adapt to low-N environments (such as natural grassland ecosystems); they also lack the ability to compete effectively with heterotrophic bacteria, which dominate in grassland systems (Woldendorp and Laanbroek, 1989). The *Nitrobacter* sequence cluster-4 replaced cluster-3 when arable land was converted to grassland (Woldendorp and Laanbroek, 1989). Subsequently, it was demonstrated that sequence cluster-4 is more adapted to low-N environments and also has better competitive ability against heterotrophs; thus it replaced sequence

cluster-3 in permanent grasslands (Woldendorp and Laanbroek, 1989).

2. Organic Matter and C/N Ratio

Nitrification in soil is influenced by the availability of ammonium ions to the nitrifiers. The quality of the SOM, especially the C/N ratio determines the heterotrophic bacterial populations and their ability to compete with the nitrifiers for NH_4^+ . Nitrifiers are poor competitors compared with heterotrophic bacteria; thus a high C/N ratio in soils usually leads to microbial immobilization of the NH_4^+ -N, effectively suppressing nitrification (Focht and Verstraete, 1977; Sahrawat, 1996). Most arable soils have a C/N ratio of about 10 where nitrification proceeds at a normal rate and immobilization of NH_4^+ -N formed is limited (Tisdale and Nelson, 1970; Vitousek *et al.*, 2002). Forest and grassland soils, where microbial immobilization of NH_4^+ -N is common, often have C/N ratios >100 (Vitousek, 1982; Vitousek *et al.*, 2002).

IV. MODES OF ACTION OF NITRIFICATION INHIBITORS

A. Biochemistry of NH_3 Oxidation

Ammonia-oxidizing bacteria (AOB, i.e., *Nitrosomonas* spp.) are predominantly chemolithoautotrophs (Bock *et al.*, 1991). They extract energy from a single inorganic source (NH_3), and assimilate inorganic substrates (i.e., CO_2 , and mineral nutrients), from which they can synthesize all essential nutrients for growth. As autotrophs, nitrifiers [i.e., AOB and nitrite-oxidizing bacteria (NOB)] typically obtain their carbon from the assimilation of CO_2 . The catabolism of ammonia (by AOB) takes place in two steps (Figure 3). Ammonia is first oxidized to hydroxylamine by ammonia monooxygenase (AMO), a copper-containing enzyme that is a membrane-bound protein (Shears and Wood, 1985; Basu *et al.*, 2003). In monooxygenase-catalyzed reactions, one atom of O from O_2 is reduced with two electrons from the substrate (NH_3 in case of AMO), usually with the insertion of the O atom into the substrate (i.e., formation of NH_2OH). There is also a requirement for additional input of reductant to reduce the second atom of O to form H_2O (Figure 3). In AOB, the reductant must come from further oxidation of the product, hydroxylamine. Hydroxylamine is oxidized to nitrite by hydroxylamine

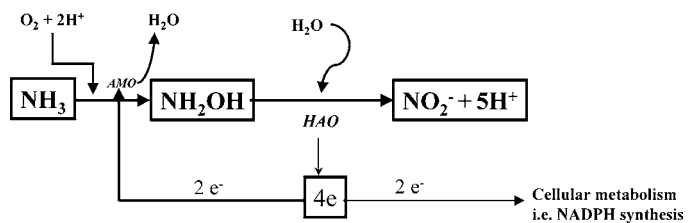


FIG. 3. Enzymatic reactions catalyzed by ammonia monooxygenase and hydroxylamine oxidoreductase in *Nitrosomonas europaea* (adapted from McCarty, 1999).

oxidoreductase (HAO). This oxidation releases four electrons, two of which are returned to AMO to sustain ammonia oxidation. The remaining two electrons are available for the cell's reductant needs (i.e., generation of NADPH) (Figure 3). Thus, AMO requires a source of two electrons that drive the reaction which come from the oxidation of the AMO reaction product by HAO (i.e., co-oxidation by HAO). This co-oxidation requirement has a substantial impact on the kinetics of alternative substrate oxidation by AMO (Hyman and Wood, 1985).

B. Inhibitory Compounds/Mechanisms Operating on *Nitrosomonas*

As shown in Figure 3, AMO and HAO enzymes play a critical role in the oxidation of NH_3 to NO_2 . Several nitrification inhibitors developed for agricultural applications target the AMO enzymatic site. Hydroxylamine, if accumulated, is toxic to *Nitrosomonas*, and thus has rarely been targeted for the development of nitrification inhibitors (Arp and Stein, 2003).

1. Ammonia Monooxygenase (AMO) Enzymatic Pathway

Monooxygenases in general have a broad substrate range, and AMO is no exception to this (Lees, 1952; Gunsalus *et al.*, 1975). Nearly 60 compounds act as alternative substrates for AMO, and can influence AMO activity in intact cells by three distinct mechanisms (Table 1):

1. Direct binding and interaction with AMO (competitive, non-competitive and metal chelators).
2. Interference with the reductant supply to AMO activity.
3. Oxidation of substrates to give products that are highly reactive and/or inactivate AMO and/or other enzymes (suicide or mechanism-based inhibitors).

Competitive inhibitors are substrates that utilize the same binding site as NH_3 . Keener and Arp (1993) proposed NH_3 -

binding sites to which competitive inhibitors bind and also an alternative binding site (such as for O_2 and another for electron donation) to which noncompetitive inhibitors bind. It has been hypothesized that the noncompetitive binding site of AMO is likely to be a hydrophobic region, which is not well-defined as indicated by the wide structural diversity of noncompetitive inhibitors affecting ammonia oxidation (Keener and Arp, 1993). Some non-competitive inhibitors may form an enzyme-inhibitor-substrate complex (Keener and Arp, 1993).

Metal chelators such as guanidine, potassium ethyl xanthate, salicylaldehyde, potassium cyanide, 8-quinolinol, sodium diethylthiocarbamate, L-histidine, 8-hydroxyquinoline, and o-phenanthroline inhibit AMO (Hooper and Terry, 1973). In particular, copper-chelators such as thiourea-based compounds (e.g., allylthiourea) inhibit AMO (Hooper and Terry, 1973; Bedard and Knowles, 1989). Inhibitors of copper enzymes such as diethylthiocarbamate and ethyl xanthate also inhibit AMO (Lees, 1946). All the substrates for AMO are uncharged and non-polar or of relatively low polarity, which indicates a hydrophobic substrate-binding pocket (Wood, 1986). It has been hypothesized that the hydrophobic nature of compounds is an important determinant of both affinity for the active site of AMO and ability to inhibit the activity of AMO. Using quantitative structure-activity relations (QSAR) modeling, Takahashi *et al.* (1997) proposed that the hydrophobicity constant (log octanol-water partition coefficient) is an important parameter for determining the inhibitory effect of triazines. It has also been hypothesized that O_2 binds to one of the metals such as Cu or Fe, which are part of the functional group of AMO in order to have the inhibitory effect.

For mechanism-based inhibitors, the ability to oxidize the compound results in the formation of a highly reactive product that can bind polypeptides and thereby inhibit catalysis of AMO. Usually this mode of inhibition involves irreversible inactivation of the enzyme through its covalent modification by the product

TABLE 1
Inhibitors and Mechanism-based (suicidal) inactivators of ammonia monooxygenase enzymatic pathway
(adapted from Arp and Stein, 2003)

Mode of action	Examples	Comments	References
I. Inhibitors			
a. Competitive vs. NH_3	Methane, ethylene, carbon monoxide	Alternative substrates	Keener and Arp, 1993
b. Noncompetitive vs. NH_3	Ethane, Propane, <i>n</i> -Butane, Chloromethane, Chloroethane, <i>n</i> -Chloropropane, Bromomethane, Bromoethane, Iodomethane, Iodoethane,	Alternative substrates	Keener and Arp, 1993
c. Metal Chelators	Thiourea, carbon disulfide, potassium cyanide	Cu-selective	Hooper and Terry, 1973; Hyman <i>et al.</i> , 1990
II. Mechanism-based (suicidal) Inhibitors	Alkynes, allylsulfide, p-anisidine	Requires enzyme turnover with O_2	Hyman <i>et al.</i> , 1988; Juliette <i>et al.</i> , 1993

of catalysis. Acetylene (C_2H_2), allylsulfide and trichloroethane (TCE) are considered as mechanism-based inhibitors (Table 1). For acetylene, the product formed by monooxygenase activity is a highly reactive unsaturated epoxide with a half-life such that it covalently binds only to the protein catalyzing the oxidation. With mechanism-based inhibition causing irreversible damage to AMO activity (i.e., loss of enzyme for *Nitrosomonas*), *de novo* synthesis of this enzyme is required for *Nitrosomonas* to resume metabolic activity (Hyman and Wood, 1985). Allylsulfide, also a suicidal inhibitor, whose oxidation product by AMO is highly unstable and rapidly binds to AMO, damages irreversibly the enzyme function (Juliette *et al.*, 1993). Several S-containing amino acids have an inhibitory effect on AMO, through the formation of volatile S compounds such as carbon disulfide (CS_2). Carbon disulfide interacts with a nucleophilic amino acid, with the formation of a stable carbonyl sulfide and covalently bound S on the microsomal membrane (Neal and Halpert, 1982). In general, compounds with internal C=S bonds and those with terminal C=S bonds form a complex with the active Cu-site of AMO (chelating function), leading to the inactivation of the enzyme (Neal and Halpert, 1982). Nitrite can inactivate AMO in the presence or absence of oxygen, although the mechanism/mode of action is presently not fully understood (Stein and Arp, 1998). The most commonly used nitrification inhibitors, nitrapyrin and DCD, act by chelating the copper components of the cytochrome oxidase involved in ammonia oxidation (Powell and Prosser, 1986).

In addition to specific inhibitors (i.e., specific to *Nitrosomonas*), several nonspecific inhibitors that affect ammonia oxidation in *Nitrosomonas* (Hooper and Terry, 1973; Hauck, 1980) have been proposed:

1. Compounds such as SKF 525 which interact with cytochrome P-450 of mammalian microsomes
2. Carbon monoxide
3. Inhibitors of catalase such as thiosemicarbazide, 3-aminotriazole, aminoguanidine, di-phenylthiocarbazon
4. Inhibitors of peroxidase such as thiosemicarbazide
5. Amine oxidases such as iproniazid
6. Uncouplers of oxidative phosphorylation such as m-chlorocarbonyl cyanide phenylhydrazone (CCCP), 2,4-dinitrophenol (DNP)
7. Electron acceptors such as phenazine methosulfate
8. Compounds such as ethyl acetate and acetone which dissolve bacterial membranes, thereby disrupting enzyme functions

2. Hydroxylamine Oxidoreductase Enzymatic Pathway

Hydroxylamine oxidoreductase (HAO) catalyzes the hydrolysis of hydroxylamine, a step in the energy-generating oxidation of ammonia to nitrite by *Nitrosomonas* (Figure 3). HAO is likely a dimer or trimer of the 63-kDa subunit (Terry and Hooper, 1981). Each subunit of MW 63 kDa (Terry and Hooper, 1981)

contains 7 c-hemes and one heme P460, with a c-heme covalently connected from a methylene carbon to a ring of a tyrosine (Arciero *et al.*, 1993); the bridging is from Cys 229 and 232 to Tyr 467 (Sayavedra-Soto *et al.*, 1994). Further, ferrous heme P460 is the only heme of HAO that reacts with exogenous small molecules where it is readily oxidized to the ferric form by dioxygen or hydrogen peroxide, and binds carbon monoxide (Logan *et al.*, 1995). Other than the substrate, the only compound known to react with the active site of ferric HAO is hydrogen peroxide, which irreversibly destroys the activity and heme P460 of HAO (Logan *et al.*, 1995). The HAO activity is inactivated by phenyl-, methyl-, or hydroxyethyl hydrazine. Also, organo-hydrazines are considered suicide substrates of HAO that directly attack the P460 active site of the enzyme (Logan and Hooper, 1995).

C. Inhibitory Compounds/Mechanisms Operating on *Nitrobacter*

Nitrite oxidation by *Nitrobacter* involves a cytochrome electron carrier system (Lees and Simpson, 1957). Oxidative phosphorylation possibly occurs within cytochrome a1 and/or cytochrome a3 as a reverse flow electron transport chain without the direct involvement of cytochrome c, yielding one ATP per nitrite mole produced (Aleem, 1970). This nitrite oxidase system functions as a respiratory complex. There is evidence that formate dehydrogenase and nitrate reductase in *Nitrobacter* use the nitrite oxidase electron transport chain and reside in the same cell particle fragment that contains nitrite oxidase (Hauck, 1980).

Chlorate is reported to suppress the *Nitrobacter* population, but not their ability to oxidize nitrite (Lees and Simpson, 1957). In contrast, cyanates, mono- and dichloro-phenyl isothiocyanate, and mercuric chloride are highly toxic to nitrite oxidase (Kinoshita *et al.*, 1966). Several pesticidal chemicals such as chlordane, CIPC, eptam, heptachlor, and lindane are reported to inhibit nitrite oxidase by depressing *Nitrobacter*, rather than by a specific effect on the nitrite oxidase system (Winely and San Clemente, 1970). Quinacrine, an inhibitor of flavoproteins, inhibits nitrite oxidation. Chelating compounds such as citrates and amines interfere with the flavoprotein-cytochrome and respiratory systems, thereby indirectly affecting nitrite oxidation (Sewell and Aleem, 1969). Uncouplers of oxidative phosphorylation such as p-nitrophenol, m-nitrophenol, 2, 4-dinitrophenol, and 2, 5-dinitrophenol are inhibitory to nitrite oxidation by *Nitrobacter* (Hauck, 1980).

V. REGULATION OF NITRIFICATION IN NATURAL ECOSYSTEMS

Since N is the limiting nutrient in many natural systems, several mechanisms have evolved to minimize N losses and effectively utilize N for survival, productivity and stability in some forest and grassland ecosystems (Vitousek and Sanford, 1986; Vitousek and Matson, 1988). Some of the mechanisms proposed include:

1. Direct absorption of organic N (such as tannin-protein complexes) or through mycorrhizal associations, thus short-circuiting the N mineralization process (Griffiths and Caldwell, 1992; Nasholm *et al.*, 1998; Kielland, 1994).
2. Inhibition of nitrification by organic compounds released by vegetation (allelo-chemicals from roots as exudates or produced in the plant tissues and added to the soil through litter), which have regulatory effects on nitrifier populations and their functioning, and on the nitrification in soils (Lata *et al.*, 1999).
3. Microbial immobilization of NH_4^+ -N through additions of carbon-rich litter, stimulating heterotrophic soil biota, thus making ammonium-N unavailable to the nitrifiers. The extent of immobilization may vary between 35 to 95% depending on the soil type and plant species (Bengtsson *et al.*, 2003).
4. Microbial immobilization of NO_3^- in certain forest and grassland ecosystems that protects NO_3^- -N from leaching and denitrification (Stark and Hart, 1997).
5. Immobilization of NO_2 through nitrosation, a chemical reaction of NO_2 with phenolics forming organic N compounds (forest and grassland soils are usually phenolic rich) (Dail, *et al.*, 2001).

Several case studies are presented to reflect the heterogeneity of natural systems in managing and regulating the nitrification, ranging from stimulation to complete inhibition.

A. Absence of NO_3^- -N in Soils of Mature Climax Ecosystems

In managed agricultural systems most of the inorganic N is in the form of NO_3^- -N (Harmsen and Van Schreven, 1955), and NH_4^+ -N is generally below detectable limits. Nitrification is very rapid in most managed agricultural systems (Huber and Watson, 1974; Sahrawat, 1980a). In contrast, most of the inorganic N found in mature natural ecosystems (such as forest and grassland) is in NH_4^+ -N form (generally ranges from 5 to 20 mg kg⁻¹) (Harmsen and Van Schreven, 1955; Jordan *et al.*, 1979). In many climax communities, NO_3^- -N is almost undetectable (Cooper, 1986; Vitousek and Matson, 1988). Successional stages of maturing ecosystems are hypothesized to have low nitrification potential, which becomes more pronounced with maturity of the climax or in long-term undisturbed ecosystems (Lodhi, 1979, 1982; Lodhi and Killingbeck, 1980; Killham, 1990; Wedin and Tilman, 1990). Such low potential climax ecosystems have relatively small nitrifier populations. Climax ecosystems of perennial grasses influence N-mineralization and nitrification; *Poa pratensis* accelerates N-mineralization, whereas *Agropyron repens* and *Andropogon gerardi* depress N-mineralization in soils (Wedin and Tilman, 1990).

The succession-based hypothesis of inhibition of nitrification has been challenged (Robertson, 1984; Stienstra *et al.*, 1994). Permanent grasslands dominated by grass species of *Holcus lanatus*, *Agrostis stolonifera*, *Anthoxanthum odoratum* and *Agrostis capillaris* did not show inhibition of nitrification.

However, grasslands dominated by *Hyparrhenia diplandra* showed a distinct influence. Depending on the ecotype of *H. diplandra*, nitrification was either stimulated or suppressed (Lata *et al.*, 1999). This also provides support for the hypothesis of Rice and Pancholy (1972) that plant species can influence nitrification in soils, either by stimulation or depression. The suppression of nitrification could provide a competitive advantage in N acquisition, and thus lead to domination in N-limited ecosystems. The ability to suppress nitrification is hypothesized to be one of the possible underlying mechanisms for *Hyparrhenia diplandra* to dominate in some of the ecosystems in West Africa and South America (Baruch and Fernandez, 1993; Lata *et al.*, 1999; 2004).

1. Forest Ecosystems

Soils under matured forest ecosystems such as coniferous forests, California Chaparral, oak/hickory forests, lodgepole pine, contain very low levels of NO_3^- -N (Cooper, 1986; Vitousek and Matson, 1988; Donaldson and Henderson, 1990a,b). Finnish coniferous forest soils generally show negligible net nitrification (Smolander *et al.*, 1995; 1998). In the Amazon rainforest ecosystem, soils show negligible nitrification and most of the mineral N is in NH_4^+ -form (Jordan *et al.*, 1979). The N input from the forest floor via litterfall in these tropical rain forests is about 85 kg N ha⁻¹ yr⁻¹. The leaching of NO_3^- -N from stream runoff is often negligible, less than 2 kg ha⁻¹ yr⁻¹. However, disturbance in these mature ecosystems leads to leaching of large amounts of NO_3^- leaching (Lodhi, 1979; Tietema, 1998). Laboratory incubation of these forest soils showed a very low nitrification potential (Jordan *et al.*, 1979). Adding NH_4^+ -N to soils did not stimulate nitrification in many cases (Smolander *et al.*, 1995; 1998) and the nitrifier populations were very small (Jordan *et al.*, 1979; Vitousek *et al.*, 1982).

The leaf leachates from various tree species were evaluated for their effect on soil nitrification and distinct species effects on soil nitrification were observed (Strauss, 2000). Leaf leachates from sugarmaple (*Acer saccharum*) trees had no effect on nitrification in incubated soil. In contrast, leaf leachates from red oak (*Quercus rubra*) and tuliptree (*Liriodendron tulipifera*) resulted in 100% inhibition of nitrification in soils (Strauss, 2000). Several mechanisms possibly operate in mature ecosystems that suppress nitrification, including:

1. Strong competition for NH_4^+ -N from the vegetation, heterotrophic microbes and in particular mycorrhizal complexes (Donaldson and Henderson, 1990a, b; Ste-Marie and Pare, 1999).
2. Nitrogen cycling within these ecosystems is dominated by a microbial N loop (i.e., N is released from SOM and incorporated into microbial biomass), preventing N from leaking. This loop is driven by plant-supplied carbon (Stark and Hart, 1997; Knops *et al.*, 2002). Competition for NH_4^+ -N by heterotrophic microorganisms is strong, as the nitrifiers are in general perceived as weak competitors for NH_4^+ -N (Vitousek *et al.*, 1982).

3. Direct suppression of nitrifiers through production and release of inhibitory compounds *via* plant litter, root exudates, and decomposing roots (Jordan *et al.*, 1979; Donaldson and Henderson, 1990a, b).
4. Fungi constitute a major component of the nitrifier community in some ecosystems such as coniferous forests (Northup *et al.*, 1995).

The relative importance of these mechanisms can vary across ecosystems where low rates of nitrification in soils have been observed. However, it appears that not all mature forest ecosystems have the ability to inhibit nitrification (Paschke, 1989; Montagnini *et al.*, 1989). Substantial levels of mineral N as NO_3^- have been observed in forests dominated by certain types of plant species (Piccolo *et al.*, 1994). Forests dominated by legume species of *Acacia mangium*, *A. auriculaiformis*, *A. mangium*, *A. confuse* and *A. holosericea*, have most of the soil mineral N in the NO_3^- -form (Paschke, 1989; Li *et al.*, 2001). Forests dominated by species of *Eucalyptus citriodora*, *Pinus elliotti* and *Schima superba*, have most of the soil mineral N in the NH_4^+ -form (Li *et al.*, 2001). The pH of all these soils is below 4.5. Soils with a pH <5.0 are not considered good for a nitrifier's growth, thus these soils would be considered to have a low potential for nitrification (Alexander, 1977). Laboratory incubations of soils from forest sites suggest that the nitrification rates in these soils are primarily influenced by the forest tree species rather than the pH of the soil (Li *et al.*, 2001). In Rondonian forest soils, terra firme forest soils in the Brazilian Amazon near Manaus (Piccolo *et al.*, 1994), Costa Rican forest soils (Amazon basin forests) (Robertson, 1984), and forests of *Pinus sylvestris* (L.), *Calluna vulgaris* (L.) Hull, and *Erica tetralix*, nitrification proceeded normally, and most of the mineral N was in the NO_3^- form. Also, leaching of a substantial amount of N took place in these ecosystems (Piccolo *et al.*, 1994).

2. Grassland Ecosystems

Mature grassland ecosystems have the ability to inhibit nitrification (Jarvis and Barraclough, 1991; Lata *et al.*, 1999). Permanent natural grasslands, which include the savannas of Africa and South America, are nutrient-poor ecosystems especially in N. Inhibition of nitrification can therefore play a key role in the functioning of these grassland ecosystems by controlling N losses (Lata *et al.*, 1999). Natural grasslands dominated by *Andropogon* are reported to contain mostly NH_4^+ -N with the near complete disappearance of NO_3^- -N as the grassland matured; this was considered to be an indication of the system's stability (Lodhi, 1979).

Natural grasslands dominated by *Brachiaria humidicola* have low NO_3^- -N and the nitrifier populations are generally lower than in soils under grasslands dominated by other *Brachiaria* species such as *B. brizantha* and *B. decumbens* or *Andropogon gayanus* (Sylvester-Bradley *et al.*, 1988). Legume pastures from the same study stimulated nitrification, and the nitrifier populations were several-fold higher than in experimental plots planted with *B. hu-*

midicola. Fertilizer applications to *B. humidicola* pastures did not cause accumulation of NO_3^- -N. Also, there was no immobilization of NH_4^+ -N; thus it was suggested that *B. humidicola* could have the ability to suppress nitrification in soils by releasing inhibitory compounds in root exudates (Sylvester-Bradley *et al.*, 1988; Ishikawa *et al.*, 2003; Subbarao *et al.*, 2005). Nitrifier populations in natural grasslands of Ghana were nearly 100-fold lower than those in improved pastures at the same location where the natural grassland was cleared and replaced with improved pasture grasses and legumes (Meiklejohn, 1968). In two climax ecosystems in tropical Costa Rica, the nitrification potential was correlated with land-use types. Nitrification potential was highest in forest soils (1.36 units), and lowest (0.19 units) in pasture (*Brachiaria* sp.) soils. This study provides another example of how plant species can influence soil nitrification potential (Carney *et al.*, 2004).

The hypothesis that plants can suppress or stimulate nitrification has been debated for many years, because of lack of convincing evidence in *in situ* studies (Stienstra *et al.*, 1994; Lata *et al.*, 1999, 2004). Recently, using two ecotypes of *Hyparrhenia diplandra* grasses (high-nitrification ecotype—HN; low-nitrification ecotype—LN), it was demonstrated that nitrification can be stimulated or suppressed depending on the ecotype of *Hyparrhenia* (Lata *et al.*, 2004). By transferring the LN ecotype from LN site to HN site (about 240 times higher rates of nitrification than that of LN site) and vice versa, it was shown that the influence on nitrification is a genetic attribute of the grass species, and not a general attribute associated with pasture grasses (Prikryl and Vancura, 1980; Lata *et al.*, 1999, 2004).

Several researchers have suggested that plants release inhibitory compounds from their roots that suppress nitrifier activity in mature grassland ecosystems (Munro, 1966; Meiklejohn, 1968; Rice and Pancholy, 1972). There have been attempts to test this hypothesis by evaluating root washings of grass species for inhibitory effect on nitrification. Moore and Waid (1971) showed that root washings of ryegrass (*Secale cereale* L.) or wheat inhibited the nitrification of NH_4^+ in a clay loam soil. The supply of NH_4^+ or other nutrients in soil were not limiting factors, and the possibility of immobilization or denitrification was also accounted for. It was concluded that the inhibitory effect on nitrification was due to root exudates. Based on extensive lysimeter studies using perennial grasses, Theron (1963) also showed that perennial grasses interfere with the nitrification by releasing inhibitory compounds from their roots. Root tissue extracts of several climax grasses showed an inhibitory effect on ammonia oxidation in pure cultures of *Nitrosomonas* (Neal, 1969; Rice and Pancholy, 1972, 1974). Robinson (1963) argued that in grassland soils, nitrification is limited by the availability of NH_4^+ -N, and found no evidence for the release of toxic substances from root exudates or from root tissues that could suppress soil nitrification. Purchase (1974) also did not find convincing evidence for inhibition of nitrification by *Hyparrhenia* root secretions.

B. The Possible Role of Allelochemicals from Plants Contributing to Reduced Nitrification in Climax Ecosystems

Plant species that dominate some of the climax ecosystems with low nitrification produce organic compounds that inhibit nitrifier activity (Likens *et al.*, 1969; Jordan *et al.*, 1979; Donaldson and Henderson, 1990a, b). These inhibitory compounds are added to the soil through decomposition of plant litter or released to the soil from the roots through exudation where they suppress nitrification (Jordan *et al.*, 1979). The degree of inhibition of nitrification appears to increase with the ecosystem's maturity with little or no nitrification occurring in some of the more mature ecosystems (Rice and Pancholy, 1972, 1973, 1974; Lodhi, 1982). It has been observed that numerous mature ecosystems, with low levels of nitrification also have low levels of nitrifiers which provide added support for this hypothesis. Among the inhibitory compounds, phenolics and terpenoids have received most of the attention.

1. Do Phenolic Compounds (from Vegetation) Inhibit Nitrifier Activity in Climax Ecosystems?

Plants produce many secondary metabolites such as phenolic acids, terpenoids, alkaloids, polyacetylenes, fatty acids, and steroids as a defense mechanism against diseases, herbivores and insects (Inderjit, 1996). Phenolic compounds that are considered potentially allelopathic are distributed throughout the plant-soil system by varied routes including leaching, volatilization, exudation from roots, and just from death and decay of plant tissues (Whittaker, 1970; Rice, 1984). Phenolic compounds at varying concentrations show a degree of antibiotic activity. The presence of hydroxyl groups increases the chemical reactivity of these molecules. Some of the phenolic compounds such as quinones possess powerful antibiotic activity (Levin, 1971). A majority of the phenolic compounds released into the soils are, however, easily degraded by soil microbes (Turner and Rice, 1975; Uren, 2000); thus, the effective bioactivity of the compounds is linked to their ability to persist in soils (Uren, 2000). Phenolic groups, particularly ortho-diphenols are susceptible to oxidation. The *O*-diphenols bind to a range of cellular receptor sites and show a range of biological activities. The type of side groups present on these compounds can determine their metal-chelating ability, and thus biological activity (Rhodes, 1985).

Phenolic acids are released into the soils in natural and managed ecosystems by root exudations or from decomposing litter (Inderjit, 1996). The total amount of phenolic compounds in soils is estimated by the total of polymers (tannins) and monomers (phenolic acids and flavonoids), which are quantitatively determined by:

1. Various chemical assays that include redox assays, metal-binding assays and assays based on specific chemical activity (Hagerman and Butler, 1991).
2. Protein-binding assays that are used to determine tannin capacity of phenolic compounds include the Folin-Denis,

Folin-Ciocalteu and Price-Butler methods (Walterman and Mole, 1994).

Assessing the importance of plant phenols in N mineralization and nitrification is further confounded by the complexity and diversity of the compounds referred to as phenolics and by the different extraction methods used (Martin and Martin, 1982; Mole and Walterman, 1987). Among available methods, extraction with 2 M NaOH is considered to give the highest recovery of the added phenolic compounds from the soil (Dalton *et al.*, 1987). The amounts of phenolic acids extracted from permanent pastures with 2 M NaOH were up to 2000 times greater (as strong alkali solutions solubilize phenolic substances that are physically and chemically bound on clay surfaces or organic matter) than the amounts extracted with water (i.e., 3000 μ M with 2 M NaOH and 1.5 μ M with water) (Whitehead *et al.*, 1981). Most of the phenolic compounds adsorbed on clay micelles, utilize binding sites close to possible *Nitrosomonas* habitat sites (Harmsen and Van Schreven, 1955). The total phenol concentration in the soil may be the preferred parameter for evaluating their influence on nitrifier activity. Also, microbial metabolism or co-metabolism of phenolic acids leads to the production of other phenolic acids by addition or deletion of side groups, which leads to changes in their biological activity (Inderjit, 1996; Blum *et al.*, 1999); this makes it extremely difficult to identify the specific allelo-chemicals released from plants.

Phenolic compounds and tannins (produced by climax vegetation) are hypothesized to reduce nitrification rates in some mature ecosystems. Phenolic acids such as *p*-hydroxybenzoic acid, *p*-coumaric acid, vanillic acid, ferulic acid, caffeic acid, ellagic acid, gallic acid, chlorogenic acid and tannins are hypothesized to suppress nitrifiers in climax ecosystems (Rice and Pancholy, 1974; Lodhi and Killingbeck, 1980). However, several researchers have challenged the hypothesis and reported that in soil incubation studies the inhibitory effects of phenolics could not be confirmed (e.g., see Bremner and McCarty, 1993).

Polyphenols in pine litter are considered responsible for the lack of nitrification in pine forest systems (Northup *et al.*, 1995). Nitrogen mineralization rates are negatively correlated with litter phenolic acid levels (i.e., tannic acid) in pine-forest soils (Figure 4a,b) (Northup *et al.*, 1995). Tannins form strong complexes with proteins which are sparingly soluble and recalcitrant to decomposition (Haslam, 1988). Some ectomycorrhizal fungi (e.g., *Amanita muscaria*) associated with conifers (e.g., *Pinus contorta*) directly utilize this organic N which results in a short-circuiting of the N cycle (Northup *et al.*, 1995). Unlike nitrate, protein-tannic acid complexes are strongly adsorbed on soil surfaces, and are largely resistant to leaching. Unlike NH_4^+ , tannin-protein complexes are not available to the nitrifiers; thus, they are not easily lost from the system (Fahey *et al.*, 1985). Tannic acid immobilizes organic-N through the formation of protein-tannin complexes (Northup *et al.*, 1995). Thus, in some pine forest ecosystems where N is a limiting factor for productivity,

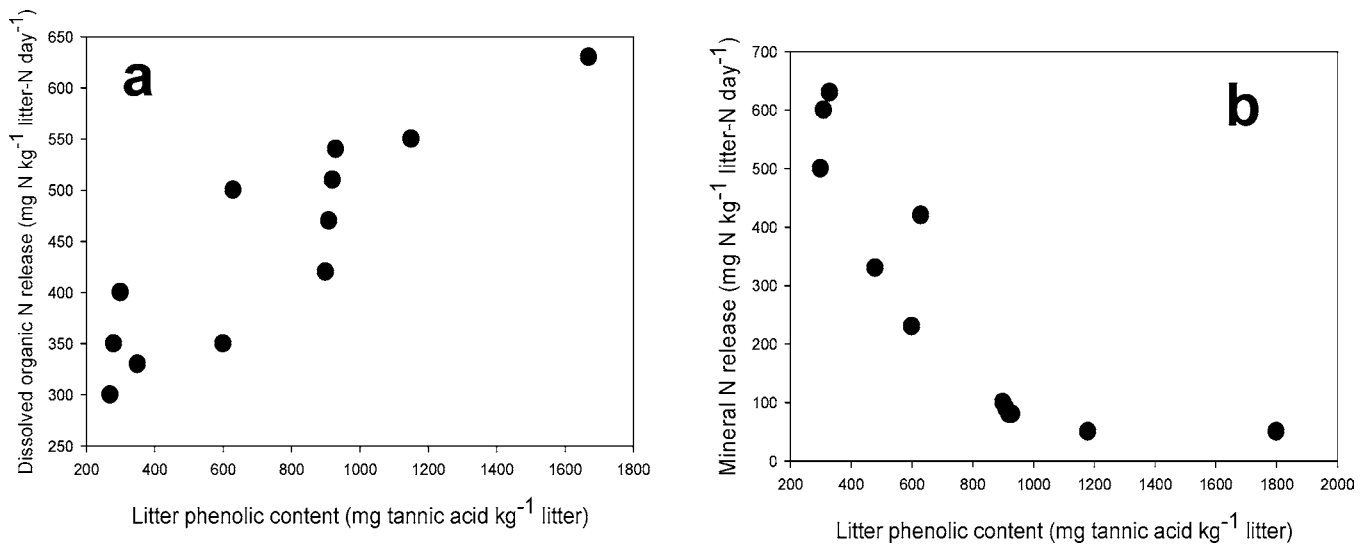


FIG. 4. (a) Release of dissolved organic nitrogen versus total phenolics of litter in *Pinus muricata* (Adapted from Northup *et al.*, 1995). (b) Release of mineral nitrogen (NH_4^+ + NO_3^-) versus total phenolics of litter in *Pinus muricata* (Adapted from Northup *et al.*, 1995).

the N contained in the litter is effectively immobilized in a form that is readily accessible to the mycorrhizae directly associated with pine root systems.

The mechanisms that operate to minimize N losses provide a comparative advantage in conserving N and maintaining long-term productivity of the mature ecosystems (Northup *et al.*, 1995). Phenolic compounds influence mineralization and ammonification of organic N (Kuiters, 1990; Hattenschwiler and Vitousek, 2000). Soluble phenols such as ferulic acid, gallic acid, and flavonoids stimulate or inhibit spore formation and hyphal growth of several saprophytic fungi (Hattenschwiler and Vitousek, 2000), thus influencing the microbial immobilization of inorganic N. Further, polyphenol production by plants in infertile soils, particularly in environments where N is limiting, may represent an adaptive attribute for microbial immobilization of inorganic N, and for minimizing nitrification and associated N losses (Kaye and Hart, 1997).

In balsam fir soils, polyphenols released from *Pinus muricata* are suggested to be the cause of low or no nitrification (Olson and Reiners, 1983). High levels of condensed tannins were found in the soils and are thought to be involved in suppressing nitrifier activity (Olson and Reiners, 1983). Similarly, tannins released from leaf litter are hypothesized to be responsible for inhibition of nitrification in tropical rain forest soils of the Amazon Basin (Jordan *et al.*, 1979). Spruce needles contain phenolic acids dominated by catechol and hydroxyacetophenone that represent more than 70% of the total phenolic acids. Concentrations of phenolic acids in these soils reach up to 13,000 mg kg⁻¹ from litter decomposition (Gallet and Lebreton, 1995). In bilberry (*Vaccinium myrtillus*) leaves, flavonoids and phenolic acids account for nearly 8% of the dry weight with caffeic acid and catechol as the main monomers. These compounds

have high levels of biological activity, including some degree of inhibition of nitrifiers (Barz and Weltring, 1985; Gallet and Lebreton, 1995). A majority of the phenolic compounds in bilberry litter are decomposed and modified with increased levels of hydroquinone (Martin *et al.*, 1979; Gallet and Lebreton, 1995). Hydroquinone inhibits nitrification in soils (Sahrawat, 1996), and is also a urease inhibitor (Hauck, 1985). Pear (*Pyrus communis*) trees produce large amounts of the glycoside arbutin and high β -glucosidase levels which are added to the soil through their litter (Hildebrand and Schroth, 1964). The enzyme β -glucosidase catalyzes the hydrolysis of arbutin to yield hydroquinone (Levin, 1971).

Phenolic compounds from the litter of *Acer saccharum*, *Fagus grandifolia*, *Betula alleghaniensis*, *Tsuga canadensis* and *Quercus rubra* forest ecosystems have effects on N cycling through multiple mechanisms (Donaldson and Henderson, 1990a,b; Hattenschwiler and Vitousek, 2000), which include N immobilization (Gallardo and Merino, 1992), and inhibitory effect on nitrifiers (Baldwin *et al.*, 1983; Fierer *et al.*, 2001). Nitrification was inhibited only in soils inhabited by red oak (*Quercus rubra*) and not in soils with the other four tree species (Lovett *et al.*, 2004). Nitrosation and microbial immobilization of NO_3^- are suggested as possible mechanisms for lack of NO_3^- formation in these ecosystems (Dail *et al.*, 2001; Lovett *et al.*, 2004).

Tannins inhibit nitrification for up to two weeks when added to the soil at 20,000 mg kg⁻¹ (Basaraba, 1964). In natural ecosystems, large amounts of tannins may be added to the soil. In some forest ecosystems, nearly 3.5 Mg litter ha⁻¹ yr⁻¹ can be added, with the average tannin levels ranging from 5 to 15% (Lutz and Chandler, 1957). Tannins added to the soil on an annual basis can have a cumulative effect over time (Kuntzel, 1955). Tannins

are toxic to a wide range of microorganisms (Scalbert, 1991; Mila and Scalbert, 1994). Some tannins, however, enhance certain microbial activity, contrary to the general perception that all tannins have antimicrobial activity (Fierer *et al.*, 2001; Lovett *et al.*, 2004). For example, tree species such as sugar maple (*Acer saccharum*) produce tannins that stimulate nitrification rates in soils (Lovett *et al.*, 2004).

2. Do Terpenoids Inhibit Nitrification in Forest Ecosystems?

Terpenoids occur commonly in conifers, composites, mints and euphorbias (Langheim, 1994). Monoterpenes, which are major constituents of many pine resin oils, have 10 carbon molecules formed by the polymerization of two isoprene units, and may be acyclic, monocyclic, bicyclic or tricyclic, and exist in both hydrocarbon and oxygenated forms (Wood, 1996). Potential sources of monoterpenes include leachate from leaf litter, canopy leaves, root exudation and deposition of volatilized monoterpenes from litter. Leaf litter and leachate are considered to be the main source of monoterpenes (Wood, 1996). Monoterpenes have anti-microbial activities as they disrupt electron transport and uncouple oxidative phosphorylation (Ward *et al.*, 1997).

Volatile terpenoids (α -terpinene, limonene, myrcene, α -pinene, β -pinene, and α -phellandrene) are abundant in pine needles, and are considered responsible for the inhibitory effect on nitrification in ponderosa pine forest soils (White, 1991; Courtney *et al.*, 1991; Fischer *et al.*, 1994; Ward *et al.*, 1997). However, studies by Bremner and McCarty (1988, 1993) reported that under laboratory conditions terpenoids did not inhibit nitrification when added to the soil. Subsequent studies showed that when soils are exposed to very high levels of terpenoids in the range of 500 to 5000 mg kg⁻¹ soil, a major portion of the NH₄⁺-N is immobilized, and the inhibitory effect is similar to adding equivalent amounts of carbon as glucose to the soil (Vitousek and Reiners, 1975).

Based on a series of experiments, Wood (1996) showed that additions of 250 to 500 mg kg⁻¹ of monoterpenes to soils had a significant and substantial inhibitory effect on nitrification; hydrocarbon monoterpenes were about twice as effective as oxygenated monoterpenes in inhibiting nitrification in soils. Using pure cultures of *N. europaea*, it was shown that terpenoids have a distinct inhibitory effect on nitrification activity (Ward *et al.*, 1997). The presence of terpenoids was thus proposed as one of the major factors for low nitrification in forest soils of coastal redwood (*Sequoia sempervirens*) and ponderosa pine (Ward *et al.*, 1997). Further, the inhibitory effect of monoterpenes on nitrification was confirmed using soil incubation studies (Paavolaianen *et al.*, 1998). The mode of inhibition of nitrification by monoterpenes appears to be in blocking of the AMO pathway which is similar to the mode of action of the commercial nitrification inhibitor nitrapyrin (Ward *et al.*, 1997).

VI. POSSIBLE STRATEGIES FOR REGULATION OF NITRIFICATION IN AGRICULTURAL SYSTEMS

A number of N-management strategies that utilize rate and/or timing of fertilizer applications such as "Fall" vs. "Spring" application, basal vs. split applications, banding of N fertilizers vs. broadcasting, deep placement of N fertilizer vs. surface application, point-injection placement of solutions, and foliar application of urea have been used to limit the availability of NH₄⁺-N to the nitrifiers. Strategies have also been developed to synchronize fertilizer application with crop N demand to facilitate rapid uptake of N and reduce the residence time of NO₃⁻-N in the soil, thus limiting denitrification and/or leaching losses (Newbould, 1989; Timmons and Baker, 1991; Dinnes *et al.*, 2002). Many of these agronomic strategies have limitations, as they are associated with additional labor costs (for split applications) and other practical difficulties (such as difficulties associated with spring fertilizer applications using heavy machinery and availability of labor and fertilizers, which are cheaper during the fall than in spring). These strategies have been discussed thoroughly (Powlson, 1993; Campbell *et al.*, 1995; Dinnes *et al.*, 2002) and are outside the scope of this review.

A. Synthetic Chemical Inhibitors

Nitrification inhibitors (NIs) are compounds that delay bacterial oxidation of NH₄⁺ by depressing the activities of nitrifiers in soil. In theory, slowing down nitrification in soils under conditions where there is a high risk of N losses either through NO₃⁻ leaching or denitrification can improve NUE (Sahrawat and Keeney, 1985). Minimizing the rate of nitrification until the primary crop is in its log phase of growth will give the plant better opportunity to absorb NO₃⁻. In addition, a rapidly growing crop may absorb most of the water from precipitation/irrigation, lowering the risk of NO₃⁻ leaching (Dinnes *et al.*, 2002).

Numerous compounds have been proposed and patented as nitrification inhibitors (Table 2) (see reviews of Slagen and Kerkhoff, 1984; Prasad and Power, 1995). Of the nitrification inhibitors listed in Table 2, only a few have been evaluated under field conditions for their effectiveness in controlling nitrification in soils, and of these only a few have been adopted to some extent in the United State, Europe, and Japan (Table 3). Only nitrapyrin and DCD have gained substantial practical and commercial importance in agricultural and horticultural crop production. 3, 4-dimethyl pyrazole phosphate (DMPP) has recently been released and recommended for large-scale adoption in Europe. The inhibitor is stated to have many advantages over nitrapyrin and DCD for the ease of application, persistence, stability and effectiveness of the inhibitory effect over longer periods under relatively higher temperatures. In the following section, we present a synthesis of the available information on the effectiveness of the most commonly used chemicals as nitrification inhibitors in agricultural systems (Table 3).

TABLE 2
Selected inhibitors of nitrification

Serial number	Name of the compound	References
1.	Nitrapyrin (2 chloro-6-(trichloromethyl) pyridine)	Goring, 1962a, b; Briggs, 1975
2.	DCD (dicyandiamide)	
3.	DMPP	Prasad and Power, 1995
4.	AM (2-amino-4-chloro-6-methylpyrimidine)	Ranney, 1978
5.	ATC (4-amino-1,2,4,-triazole hydrochloride)	Guthrie and Bomke, 1980
6.	Cl 1580 (3,4-diamino-6-trichloromethyl-s-triazine)	See McCarty, 1999
7.	ASU (1-amido-2-thiourea)	
8.	MT (3 mercapto-1,2,4-triazole)	
9.	MAST (2-amino-4-methyl-6-trichloromethyltriazine)	
10.	ST (2-sulfanilamidothiazole)	Mitsui Toatsu, 1968
11.	DCS (N-2,5,-dichlorophenyl succinamide)	Mosier <i>et al.</i> , 1996
12.	MPC (3-methyl-pyrazole-1-carboxamide)	McCarty and Bremner, 1989
13.	CS ₂	Ashworth <i>et al.</i> , 1977
14.	CP (2-cyanimino-4-hydroxy-6-methylpyrimidine)	
15.	ATS (Ammonium thiosulfate)	Goos, 1985
16.	AMP (Ammonium polycarboxylate)	
17.	CCC/ECC (Wax coated/encapsulated calcium cabide)	Freney <i>et al.</i> , 1993
18.	Dwell (etridiazole)	Varsa <i>et al.</i> , 1981
19.	Sodium thiocarbonate	Hauck, 1980
20.	ST (Sodium thiosulfate)	
21.	Thiourea	see Prasad <i>et al.</i> , 1971.
22.	ZPTA (Thiophosphoryl triamide)	Radel <i>et al.</i> , 1992
23.	Isothiocyanates	Slangen and Kerkhoff, 1984
24.	Nitro and haloanilines	Slangen and Kerkhoff, 1984
25.	Xanthates	Slangen and Kerkhoff, 1984
26.	Potassium azide	Hughes and Welch, 1970
27.	Thioacetamide	Hauck, 1980
28.	Sodium thiocarbamate	Hauck, 1980
29.	Thiosemicarbazide	Hauck, 1980
30.	Diphenylthiocarbazon	Hauck, 1980
31.	Dithiocarbamate	Hauck, 1980
32.	s-ethyl dipropylthiocarbamate	Hauck, 1980
33.	Ethylene-bis-dithiocarbamate	Hauck, 1980
34.	Ethylene urea	
35.	N-methyldithiocarbamate	Hauck, 1980
36.	Sodium diethylthiocarbamate	Bundy and Bremner, 1973; Hauck, 1980
37.	Phenyl mercuric acetate	
38.	Sodium-diethyl-di-thiocarbamate	
39.	2-ethynylpyridine	McCarty and Bremner, 1986
40.	3-Methylpyrazole-1-carboxamide	
41.	C ₂ H ₂ (acetylene)	Berg <i>et al.</i> , 1982
42.	Phenylacetylene	McCarty and Bremner, 1986
43.	Propyne	McCarty and Bremner, 1986
44.	1-Butyne	McCarty and Bremner, 1986
45.	3-Butyne-2	McCarty and Bremner, 1986
46.	C ₂ H ₆ (ethane)	McCarty and Bremner, 1991
47.	3-chloro-acetanilide	
48.	2,5-dichloro-aniline	
49.	4-Amino-1,2,4-triazole	Bundy and Bremner, 1973

TABLE 2
Selected inhibitors of nitrification (*Continued*)

Serial number	Name of the compound	References
50.	N-(2,5-Dichlorophenyl) succinamide	
51.	2-Mercaptobenzothiazole	Hauck, 1980; McCarty and Bremner, 1986
52.	2-mercapto-1,2,4-triazole	Bundy and Bremner, 1973
53.	3-mercapto-1,2,4-triazole	Hauck, 1980
54.	2,5-dichloroaniline	Bundy and Bremner, 1973
55.	5-ethoxy-3-trichloromethyl-1-1,2,4-thiadiazole	
56.	o-nitrophenol	Topalova <i>et al.</i> , 1995
57.	m-nitroaniline	Hoeflich, 1968
58.	o-nitroaniline	Hermann <i>et al.</i> , 1967
59.	Benzotriazole	McCarty and Bremner, 1989
60.	Pyrazole	McCarty, 1999
61.	1,2,4-Triazole	McCarty, 1999
62.	Pyridazine	McCarty, 1999
63.	Indazole	McCarty, 1999
64.	N-O-furfural oxime ethers and furfural Schiff bases	Datta <i>et al.</i> , 2001

1. Nitrapyrin

Nitrapyrin was developed by the Dow Chemical Company and marketed under the trade name ©N-Serve as a nitrogen stabilizer. Nitrapyrin [2-chloro-6-(trichloromethyl)-pyridine] is soluble in organic solvents (such as acetone, ethanol, toluene, xylene and methylene chloride) and anhydrous ammonia (Slangen and Kerkhoff, 1984). Nitrapyrin has been extensively used as N stabilizer in North America, where it is injected directly into the soil mixed with anhydrous ammonia as the N carrier (Slangen and Kerkhoff, 1984). Since nitrapyrin has a relatively high vapor pressure (Table 3), it needs to be incorporated into soil at a depth of at least 5 to 10 cm (McCall and Swann, 1978). Being volatile, nitrapyrin is not effective as a coating for solid N fertilizers that are broadcast (Slangen and Kerkhoff, 1984).

Using pure cultures of *Nitrosomonas europaea*, it was shown that Nitrapyrin at a concentration of 1.0 mg kg⁻¹ is effective

in inhibiting nitrification (Zacheri and Amberger, 1990). Also, nitrapyrin does not have an inhibitory effect on the *Nitrobacter* group of soil bacteria. The inhibitory action is specific to the *Nitrosomonas* group of bacteria (Goring, 1962a,b). Based on laboratory incubation studies, nitrapyrin at 2 mg kg⁻¹ soil was effective in inhibiting nitrification for 6 weeks in 74 out of 87 soils tested (Goring, 1962a,b). Several researchers have shown that nitrapyrin at concentrations ranging from 1 to 10 mg kg⁻¹ soil is effective under laboratory soil incubation studies in inhibiting nitrification (see review by Prasad *et al.*, 1971). However, nitrapyrin was found to be ineffective in controlling nitrification in organic soils (Histosols) (Sahrawat *et al.*, 1987).

Decomposition of nitrapyrin in soil is normally complete within 30 days in warm soils that are conducive to crop growth. However, nitrapyrin is very persistent and stable in cool soils; thus, it can effectively be used with fall or winter N fertilizer applications. Autumn application of nitrogen fertilizers without

TABLE 3
Commonly used nitrification inhibitors in agriculture

Name (chemical, trademark)	Solubility in water(g L ⁻¹)	Relative volatility	Mode of application
2-chloro-6-(trichloromethyl) pyridine. (Nitrapyrin; N-Serve)	0.04 (at 20C)	High	Suitable with anhydrous ammonia with injection into the soil
2-amino-4-chloro methyl pyrimidine	1.25 (at 20C)	High	Coatings on solid nitrogen fertilizers
Dicyandiamide, cyanoguanidine, DCD	23.0 (at 13C)	Low	Blending with urea or other solid nitrogen fertilizers
DMPP		Low	Blend with urea or other solid nitrogen fertilizers

nitrification inhibitor (such as nitrapyrin) results in heavy losses of N (through NO_3^- leaching and denitrification) in light-textured and waterlogged soils. In certain environments, such as autumn-planted corn and winter wheat in North America, nitrapyrin is effective in inhibiting nitrification; its application improves N recovery and reduces N losses by NO_3^- leaching and denitrification (Warren *et al.*, 1975; Killorn and Taylor, 1994). The N dosage to autumn-planted corn can be reduced when nitrapyrin is applied because of the reduced N losses (Christensen and Huffman, 1992). Nitrapyrin has been evaluated extensively in the field for various crops and under different climatic conditions (see reviews of Rodgers, 1986; Slangen and Kerkhoff, 1984; Prasad and Power, 1995).

2. Dicyandiamide (DCD)

Dicyandiamide (DCD) was discovered as a nitrification inhibitor in the early 1920s (see Prasad *et al.*, 1971). DCD is soluble in water, non-volatile (unlike nitrapyrin) and suitable for use as coatings on solid nitrogenous fertilizers such as urea, and ammonium sulfate or for incorporation with solid nitrogenous fertilizers as a stabilizer. In soil, DCD is decomposed and converted via guanyle urea and guanidine to urea, a conventional fertilizer (ODDA, 1995). DCD has a specific bacteriostatic effect on *Nitrosomonas* (i.e., the bacteria are not killed, but only the biological activity is suppressed), and has been classified as a slow-release fertilizer (Sturm *et al.*, 1994). DCD is incorporated into conventional ammonium-containing fertilizers at a rate of 5 to 10% DCD-N (Slangen and Kerkhoff, 1984). DCD is also suitable as a stabilizer for cattle manures and animal slurries (Amberger, 1989; Dittert *et al.*, 2001). Using pure cultures of *Nitrosomonas*, the inhibitory effect of DCD was demonstrated; however, a rate of 200 mg kg^{-1} was needed to match the inhibitory effect of nitrapyrin at 1 mg kg^{-1} (Amberger, 1989). About 10 to 50 mg kg^{-1} of DCD are normally required to inhibit nitrification, and the inhibitory effect usually lasts between 4 to 8 weeks, depending on temperature, water content, organic matter and pH of the soils (Amberger, 1989, 1993). One of the major limitations of DCD is that it easily leaches out of the rooting zone, lowering its effectiveness (McCarty and Bremner, 1989). DCD, being non-volatile, is relatively more suitable for tropical climates. DCD is phytotoxic at a rate of 20 mg kg^{-1} or higher to crops such as cotton, corn, sorghum and potato (Prasad and Power, 1995). DCD does not have an inhibitory effect on general biological activity (i.e., the heterotrophic activity of the soil) other than nitrification (Amberger, 1989). The effectiveness of DCD as a nitrification inhibitor in reducing NO_3^- leaching, N_2O emissions and improving NUE has been tested extensively for various crops, and various climatic conditions (Amberger, 1989; Di and Cameron, 2002, 2004).

3. 2-amino-4-chloro-6-methylpyrimidine (AM)

AM is a white crystalline substance, soluble in water, and anhydrous ammonia. AM is not volatile; thus, is suitable for use as a coating on solid N fertilizers at temperatures of up to

35°C (Raney, 1978). Additions of 4 to 10 mg kg^{-1} of AM are effective in controlling nitrification in soils for about 20 days to one month (Slangen and Kerkhoff, 1984). Like nitrapyrin, AM has bactericidal effect on *Nitrosomonas* (Prasad *et al.*, 1971). About 5 to 6 kg of AM ha^{-1} is recommended for controlling nitrification (Prasad *et al.*, 1971). Only limited field evaluations have been made for a few crops to test the effectiveness of AM as a nitrification inhibitor (Slangen and Kerkhoff, 1984).

4. DMPP

3, 4-Dimethylpyrazol-phosphate, a newly developed nitrification inhibitor by BASF (Germany), is considered highly specific in inhibiting nitrification over a period of 4 to 10 weeks at rates of 0.5 to 1.0 kg of the active compound ha^{-1} (Zerulla *et al.*, 2001). The duration of effectiveness varies depending on the soil and climatic conditions, and also to some extent on the crop (Zerulla *et al.*, 2001). Like other nitrification inhibitors such as nitrapyrin and DCD, DMPP is persistent and effective in inhibiting nitrification at 5°C; however, at 20°C the inhibitory effect from DMPP lasts only for 40 days (Zerulla *et al.*, 2001). In contrast to DCD, DMPP is relatively immobile in the soil and stays close to where the NH_4^+ -N is adsorbed, and thus is more effective in inhibiting nitrification (Azam *et al.*, 2001; for review see Pasda *et al.*, 2001). Extensive field evaluations have demonstrated that DMPP application reduces nitrification, NO_3^- leaching, and has a positive impact on biomass and grain yields (Weiske *et al.*, 2001).

B. Factors Influencing the Effectiveness of NIs

A number of physical, chemical and biological factors determine the effectiveness of NIs in the field. To be effective, compounds proposed as nitrification inhibitors must retain both persistence and bioactivity in the field. Some of the factors influencing the effectiveness of NIs are listed in Table 4. In general, NIs are more effective in light-textured soils, soils low in organic matter and at low temperatures, but less effective in heavy-textured soils, soils with high OM, and at high temperatures (Sahrawat, 1980b; Sahrawat and Keeney, 1985).

1. Inhibitor Properties

Nitrification inhibitors differ in water solubility and volatility which in turn affects their mobility, persistence, and effectiveness. The ideal inhibitor should have about the same mobility in the soil as that of NH_4^+ -N, but this is not the case with most nitrification inhibitors. For nitrapyrin, the mobility is lower than that of NH_4^+ -N, and the mobility of DCD is several-fold higher than that for NH_4^+ -N, thus limiting their effectiveness (Pasda *et al.*, 2001). For DMPP, however, the relative mobility is reported to be about the same as that of NH_4^+ -N (Pasda *et al.*, 2001). Gaseous inhibitors such as CS_2 and C_2H_2 are highly volatile and disperse rapidly through soil pores, and become effective more rapidly than the less volatile inhibitors (such as DCD, DMPP), but they are less persistent. Their inhibitory effect generally does not last

TABLE 4
Factors that determine the effectiveness of nitrification inhibitors in soils (Adapted from Keeney, 1986)

Serial number	Factor	Remarks
I.	Inhibitor properties	
a.	Water solubility	Determine the mode of application, leaching of the inhibitor
b.	Volatility	Determine the movement in soil, and also effectiveness at high temperatures
c.	Sorption on colloids (particularly on OM or clay)	Limits the rate of leaching and mobility in soil
d.	Stability (rate of degradation)	Persistence of the inhibitor in soils
II.	Soil chemical and physical properties	
a.	pH	Affects stability and solubility of the inhibitor; also affects nitrifier activity upon which the inhibitor is expected to show its affect
b.	Organic matter (OM) levels	Sorption of the inhibiting compound, affects mobility and stability of the inhibiting compounds
c.	Porosity	Affects oxygen levels in soils which determine the nitrifier activity; also, determine the effectiveness of inhibitors that have high volatility
d.	Soil N from mineralization of OM	NH_4^+ -N produced through soil mineralization may not be accessible to the inhibitor to prevent nitrification
III.	Soil biological properties	
a.	Nitrifier populations	The biological activity of the nitrifier population will also determine the effectiveness of the inhibitor; in soils, that have very high rates of nitrification, higher inhibitor concentrations are needed to control; also, genetic diversity of <i>Nitrosomonas</i> strains, which may vary their sensitivity to the nitrifiers thus can modulate the inhibitory effect depending on the genetic make up of the nitrifer population in a given region.
b.	Soil carbon levels	May determine the heterotrophic microbial activity in soils, will influence the rate of NH_4^+ microbial immobilization. Also, heterotrophic microbial populations may metabolize, thus decompose the inhibitor compounds, and limit their persistence in soils.
IV.	Abiotic factors	
a.	Temperature	Nitrifier activity is temperature dependent, lower temperatures (such as winter season) usually have low nitrifier activity, thus inhibitors are effective; as the soil temperatures increase to above 15°C and more (usually spring and summer), nitrifier activity will increase, thus more difficult for the inhibitors to control nitrification. Also, many inhibitors are physically and biological unstable at temperatures at 15°C and above, their effectiveness decreases linearly with an increase in temperature.
b.	Water status	Determines the nitrifier activity and the movement of inhibitor in the soil. It also affects aeration.
V.	Other factors related to fertilizer type and mode of application	
a.	Type of fertilizer (i.e., ammonium sulphate or urea)	Fertilizer influence soil pH as ammonium sulfate usually results in acidic pH and urea-N results in alkaline pH.
b.	Mode of application (i.e., banding vs. broadcasting)	NIs application as a band on to the banded fertilizer is usually more effective than when the inhibitor is broadcasted along with the fertilizer, but requires higher concentrations of the inhibitor.

long enough to be significant in the field (Ashworth *et al.*, 1977; Touchton *et al.*, 1978). In organic soils such as histosols, nitrapyrin is adsorbed and not effective (Sahrawat *et al.*, 1987). In general, organic inhibitors are strongly adsorbed by SOM which reduces their mobility, bioactivity and effectiveness, but at the same time SOM adsorption enhances their persistence (Keeney, 1986).

2. Soil Chemical and Physical Properties

In general, soil pH is one of the important factors determining the biological activity of nitrifiers and heterotrophs. Inhibitors such as AM are chemically unstable at soil pH ≤ 4 , and thus are not suitable for acidic soils (Toyoda *et al.*, 1978). Nitrapyrin is stable in the pH range of 2.7 to 11.9 (Keeney, 1980; Slangen and Kerkhoff, 1984), but less effective at soil pH > 6.5 and at high levels of SOM (Hendrickson *et al.*, 1978; Touchton *et al.*, 1979). The rate of nitrapyrin required for effective inhibition of nitrification in soils increases with soil pH and SOM (Goring, 1962a; Keeney, 1986). This is largely due to rapid hydrolysis of nitrapyrin at high pH and adsorption of nitrapyrin on the SOM (Briggs, 1975). Similarly, DCD decomposes rapidly in sandy loam soils with relatively high organic matter content (Slangen and Kerkhoff, 1984). This is mostly due to the utilization of DCD-N by heterotrophic microorganisms.

Soil texture and SOM influence the effectiveness and persistence of nitrification inhibitors (Slangen and Kerkhoff, 1984). Sorption of inhibitor by the SOM reduces its mobility, volatility, bioactivity, and thus their effectiveness (Keeney, 1986). In contrast, adsorption of NIs is less in light soils with low SOM ($\leq 1\%$) and they remain bioreactive and effective. Nitrapyrin and other inhibitors are more effective in light-textured soils with low organic matter levels ($\leq 1\%$) than in heavy-textured and soils high in organic matter (Slangen and Kerkhoff, 1984; Gilmour, 1984; Sahrawat and Keeney, 1985). For example, nitrapyrin completely inhibits nitrification in soils with 1% SOM, but loses its effectiveness in soils with 5% SOM (Hendrickson and Keeney, 1979).

3. Soil Biological Properties

Genetic variability in *Nitrosomonas* strains and differences in sensitivity to NI could be one of the factors governing NI's effectiveness. Strains of *Nitrosomonas* sp. showed remarkable differences in sensitivity to nitrapyrin (Belser and Schmidt, 1981). Certain strains of *Nitrosomonas europaea* oxidize nitrapyrin to 6-chloropicolinic acid, rendering the inhibitor less effective (Vannelli and Hooper, 1992). Similarly, s-triazine and nitrapyrin have differential inhibitory effects on two strains of *Nitrosomonas*—ATCC 25978 and TK 794, where the former is relatively less sensitive to both the inhibitors.

4. Abiotic Factors

Soil temperature has a profound effect on the persistence of many NIs. The rate of nitrification increases linearly with temperatures, reaching a maximum at about 30°C in most soils.

Most NIs including nitrapyrin, DCD, AM, and DMPP are very effective at $\leq 5^\circ\text{C}$. Under low temperatures, the inhibitory effect can last up to six months, making inhibitors suitable for applications in fall and winter (Slangen and Kerkhoff, 1984; Zerulla *et al.*, 2001). However, as the temperatures increase above 10°C, there is a linear decrease in the effectiveness of most of the nitrification inhibitors; at temperatures of $\geq 25^\circ\text{C}$, the inhibitory effect lasts only two to three weeks at the most (Touchton *et al.*, 1979; Zerulla *et al.*, 2001). The inverse relationship between soil temperature and inhibitor effectiveness is largely due to less persistence of many inhibitors in the soil, and increased biological activity of nitrifiers at higher temperatures (Gomes and Loynachan, 1984). The half-life for nitrapyrin in the soil is 43 to 77 d at 10°C, but only between 9 to 16 d at 20°C (Slangen and Kerkhoff, 1984). Similarly, DCD degradation and efficacy is a function of soil temperature (Vilsmeier, 1980). The inhibitory effect of DCD lasted up to 80 days at a soil temperature of 8°C, but only for 20 to 40 days when the temperatures increased to 20°C (Vilsmeier, 1980). The inhibitory effect of DCD and DMPP lasted for about 3 months at a soil temperature of 10°C, but only for a week at a soil temperature of 30°C (Irigoyen *et al.*, 2003). Nitrification inhibitors, including nitrapyrin and DCD, become unstable and less effective as the soil moisture increases (Hendrickson and Keeney, 1979; Vilsmeier, 1980). Hydrolysis and volatilization of nitrapyrin is more pronounced in saturated soils than in aerobic soils (Slangen and Kerkhoff, 1984). Generally NIs are more effective at soil moisture contents lower than field capacity (Rice and Smith, 1983).

C. Nitrification Inhibitors (NIs)—Nitrogen-Use Efficiency (NUE) and Grain Yield Improvements

Yield increases in response to N fertilizer, are in general greater on irrigated coarse-textured soils, but losses of N (particularly NO_3^- leaching) are also significant under these conditions (Malzer, 1979). Several studies with nitrapyrin, DCD and DMPP on winter wheat, corn, rice, grain sorghum, potato, sugar-beet, and cotton have demonstrated that NIs suppress nitrification, improve N recovery and increase the economic yields significantly (see review by Prasad and Power, 1995).

D. Nitrification Inhibitors—Other Perspectives

The inhibition of nitrification by NIs indirectly influences the chemical composition of the edible portions of the plant and the emission of greenhouse gases such as N_2O (Sahrawat and Keeney, 1984; Sahrawat, 1989). Also, by changing the relative amounts of $\text{NH}_4^+/\text{NO}_3^-$ available in the soil, addition of NIs can have favorable biological effects on plant growth:

1. Rhizosphere pH may be acidified and this may impact the availability of certain nutrients such as P and Fe when soil pH is high (Gentry and Below, 1993).
2. Less metabolic energy needed to assimilate NH_4^+ -N compared with NO_3^- -N (Salsac *et al.*, 1987; Pasda *et al.*, 2001).

3. Improved uptake of N because of differential absorption of NH_4^+ -N and NO_3^- -N by crops. For example, in wheat, N uptake was increased by 35% when 1/4 of the total N was supplied as NH_4^+ compared with that when all N was NO_3^- (Wang and Below, 1992). High-yielding corn genotypes did not absorb NO_3^- during ear development, but did take up and assimilate NH_4^+ -N. Thus NIs can improve the total N uptake because of the plant's differential requirement for N forms which may vary depending on growth stage and crop (Pan *et al.*, 1984).

1. Influence on Various Components of the Nitrogen Cycle

NIs can directly or indirectly influence other N cycling processes in soils:

1. N transport and movement
2. Ammonium fixation and release
3. Ammonia volatilization and urea hydrolysis
4. Mineralization and immobilization
5. Nitrous oxide production
6. Denitrification

In some soils NH_4^+ is strongly fixed by soil minerals and may not be available to a growing crop at times of critical demands (Aulakh and Rennie, 1984). In calcareous soils suppression of nitrification and accumulation of higher levels of NH_4^+ -N may lead to increased losses by volatilization, especially from surface applied N (Sahrawat, 1989). By increasing the persistence of NH_4^+ -N in soils, NIs may accelerate N immobilization, as NH_4^+ is the preferred form of N for many soil microorganisms (Juma and Paul, 1983). The production of N_2O is an integral part of the nitrification pathway; by suppressing nitrification, NIs can also reduce N_2O production during nitrification and in subsequent conversions *via* denitrification (Sahrawat and Keeney, 1986).

2. Disease Resistance in Crops

The form of N (i.e., NH_4^+ or NO_3^-) absorbed and assimilated by plants may influence the severity of bacterial or fungal diseases. Ammonia-N has a fungicidal activity against some pathogens (Huber and Watson, 1974). Nitrification inhibitors can indirectly affect crop growth by influencing disease development and host resistance where NH_4^+ suppresses the disease-causing bacteria or fungi. Propagules of *Fusarium roseum* and *F. oxysporum* were more rapidly destroyed in the laboratory with NH_4^+ -N rather than NO_3^- -N (Smiley *et al.*, 1972). Root rot of corn, cotton, and scab of potato are decreased when plants absorb NH_4^+ -N (Huber and Watson, 1974). Retaining N in NH_4^+ -form reduced the severity of at least three root diseases of wheat *viz.*, *Cercospora* (eyespot), *Gaeummaromyces graminis* (take all) and *Fusarium* root rot (Papendick and Cook, 1974); these three diseases were controlled when nitrapryin was applied to suppress nitrification (Papendick and Cook, 1974). Similarly, stalk-rot incidence in corn was suppressed by NH_4^+ -N (Warren *et al.*, 1975). In contrast, NH_4^+ -N accelerated the incidence of

root rot in corn (Huber and Watson, 1974). Likewise, for some crops such as soybean and potato, NH_4^+ -N increases disease severity (Huber and Watson, 1974).

3. Food Quality

The availability of N as nitrate results in increased tissue concentration of nitrate in many plants. By suppressing nitrification in soils, NIs can reduce the nitrate availability in soils, thus limiting its uptake and subsequent storage in plant tissues (Barker and Mills, 1980; Sahrawat and Keeney, 1984). Application of NIs can substantially reduce NO_3^- levels in the edible portions of several horticultural crops (such as lettuce, radish, spinach, and tomato) (Irigoyen *et al.*, 2003).

4. Energy Conservation

Inorganic N fertilizers are produced from anhydrous ammonia obtained by industrial fixation of atmospheric N; the process requires the expenditure of considerable amounts of energy ($13,800 \text{ kcal kg}^{-1} \text{ N fixed}$) (Viets, 1975). Worldwide, nearly 100 million Mg N fertilizer is currently used, thus requiring substantial amounts of energy consumption. In addition, energy is spent during transport, application and incorporation of N into fertilizers. Utilization efficiency of N fertilizer in agricultural systems rarely exceeds 50%, often not exceeding 40%. By using appropriate NIs, it is possible to substantially reduce N losses associated with the nitrification in soils. This would improve N recovery and reduce N fertilizer requirement and its use. The current N fertilizer recommendations take into account the N losses that occur during and following nitrification. The N losses could be reduced substantially (up to 30%) in specific situations by using NIs. This would result in savings in energy inputs into agricultural systems which is in addition to the energy savings from decreased amounts of N fertilizer requiring transport from point of manufacture to point of use. Fall application of stabilized N fertilizers to wheat gave similar or higher yields than spring application and/or split applications of N fertilizers (Nelson and Huber, 1980). Energy savings could also result from elimination of split applications of N fertilizers that are necessitated by high N loss of the basal fertilizer applications (Nelson and Huber, 1980). Fall application of N fertilizers will also result in significant energy savings because of improved distribution of fertilizer plus more efficient handling, storage and use of application equipment (Nelson and Huber, 1980).

E. Slow- and Controlled-Release Nitrogen Fertilizers

Slow- and controlled-release fertilizer (SCR) refers to a type of N fertilizer that delays N availability for plant uptake or extends its availability to plants relative to "normal available N fertilizers" such as ammonium nitrate, ammonium sulfate, ammonium chloride, and urea (AAPFCO, 1997). SCR fertilizers release N into the soil solution at a controlled and slow rate. The general principle is that controlled or slow N release can be

achieved through special chemical and physical characteristics; conventional soluble fertilizer materials are given a protective coating or encapsulation (water-insoluble, semi-permeable or impermeable with pores), which controls water entry and rate of dissolution and nutrient release so that release is more synchronized with the plant's needs (Fujita *et al.*, 1992). These specialized nitrogenous fertilizers are less soluble in water and release N slowly such that it remains in the root zone for extended periods of time (for detailed discussion, see review by Prasad *et al.*, 1971). Because of the slow release of N to the soil, the availability of NH_4^+ -N to the nitrifiers is limited and they have to compete for N with the growing crop.

Field evaluation of polymer-coated urea (POCU) indicated that N losses associated with the nitrification could be substantially reduced, along with a concurrent improvement in N recovery (Shoji and Kanno, 1994). Because of the reduced N losses, the N requirement from POCU is about 40% less than the recommended levels of normal fertilizers (Balkcom *et al.*, 2003; Zvomuya *et al.*, 2003). Recovery of basal N application ranged from about 22% with conventional broadcast application of ammonium sulfate or urea to about 79% with co-situs application of POCU (Shoji and Kanno, 1994). However, POCU may leave undesired residues of plastic in the fields (up to $50 \text{ kg ha}^{-1} \text{ yr}^{-1}$) (Shoji and Kanno, 1994). Another important limitation for POCU is the cost, which is about 4 to 8 times the cost of normal urea (Landels, 1991; Detrick, 1996). Thus, increased acceptance of POCU for agricultural uses depends on continued technological and conceptual advances in production and realization of potential agronomic and environmental advantages, offered by new technologies in relation to the development of POCU (Shaviv and Mikkelsen, 1993).

F. Limitations/Constraints for Lack of Widespread Adoption

The cost of NIs (nitrapyrin or DCD) is about US\$ 25-35 ha^{-1} , which amounts to about 25 to 30% of the cost of N fertilizer. Thus, for NI application to be economically profitable, the long-term average losses must exceed 40 to 50 kg N ha^{-1} (Nelson and Huber, 1980). In addition, several studies indicate lack of consistency in the performance of NIs in improving yield and nitrogen-use efficiency. Some crops/cultivars do not perform well when fed with high levels of NH_4^+ , and NIs may not be suitable for such crops (Sahrawat, 1980b). Nitrification is fast in warmer temperatures and at higher soil pHs; however, currently available NIs are not effective under these conditions (Sahrawat, 1980b; Sahrawat and Keeney, 1985).

The performance of NIs has not been consistent over the years, or among locations. This has led to lack of widespread adoption even in niche production systems where they effectively control nitrification (Trenkel, 1997). In the United States, nearly 1.82 million ha are treated with either nitrapyrin or DCD which amounts to about 1.16% of the cropped area. In Western Europe, DCD-containing N fertilizers are applied only to about

200,000 ha of agricultural land, amounting to about 0.29% of the total area under cultivation (Trenkel, 1997). However, as producers begin to understand the multiple benefits of NI use in crop production which include yield increases, improved crop quality, disease control, management flexibility, reduced pollution and decreased energy consumption, their use might become more popular. When a new cost-effective generation of NIs becomes available, their adoption should become more widespread.

The main obstacle for SCRs and in particular POCU is their cost (8 times the cost of normal urea) (Goertz, 1993). Currently only about 500,000 Mg POCU is produced and applied to agricultural systems in Japan, and this amounts to only about 0.15% of the world's total N fertilizer consumption (Trenkel, 1997). Unless some of the stated bottlenecks are removed through the development of a new generation of NIs and SCR that can effectively control nitrification under temperate and tropical conditions in a cost-effective manner, NIs and SCR use will not be widely adopted. Furthermore, if legislation places restrictions on N fertilizer applications in sensitive agricultural regions where there is potential for NO_3^- leaching and polluting water bodies, farmers may opt for innovative N fertilizers that reduce nitrification. The extent of adoption by farmers depends on the availability of next generation technologies that are more functional and cost effective in controlling nitrification.

VII. PERSPECTIVES FOR BIOLOGICAL REGULATION OF NITRIFICATION IN AGRICULTURAL SYSTEMS—A VIEWPOINT

Intrinsic NUE at the physiological level is difficult to change in crops, and perhaps agronomically irrelevant. The best strategy for improving agronomic NUE (i.e., dry matter produced per unit of applied N) would be to improve N recovery by extending the time of availability of the applied N. Suppressing nitrification and keeping N in NH_4^+ form for extended periods is one way of improving recovery and agronomic NUE in agricultural systems. As shown in earlier sections of this review, nitrification has a profound impact on N recovery and utilization in agricultural systems. Plants stimulate or suppress microbial activity in the rhizosphere through release of various organic compounds as root exudates (Baath *et al.*, 1978; Van Veen *et al.*, 1989; Liljeroth *et al.*, 1990; Parmelee *et al.*, 1993). Surprisingly, little attention has been given to understanding the role of the crop plants on the process of nitrification and inherent differences among crops in regulating nitrification. With the exception of the last two centuries major food crops have evolved under natural systems and may have built up several genetic/physiological mechanisms that influence soil nitrification.

The differences in nitrification capacity of soils from various ecosystems cannot be explained solely on the basis of soil physical or chemical characteristics (Clark *et al.*, 1960; Keeney, 1980; Montagnini *et al.*, 1989; Wedin and Tilman, 1990; Lata *et al.*, 2004). Often the influence of vegetation in determining the nitrification ability of soils is not known and ignored or relegated

to 'black box' status (Clark, 1962; Donaldson and Henderson, 1990a, b). When specific mechanisms (such as the release of organic compounds that have a direct inhibitory effect on nitrifiers) are identified and their genetic control is established in plant species found in natural ecosystems, it should be possible to transfer these specific genetic mechanisms to major food and fiber crops. In addition, we speculate that various mechanisms exist in the case of major food crops (at least in some of the old land-races and wild relatives of major field crops) for the regulation of nitrification (inhibition or stimulation). These mechanisms need to be understood and better characterized before it is feasible to incorporate them into major field crops. Discovery and genetic exploitation of these novel biological mechanisms should be a major focus of crop improvement efforts in the near future. Understanding and characterizing the nitrification inhibition ability of major field crops is critical in assessing the potential for genetic exploitation, both from a cropping systems and crop improvement perspective. Current research efforts suggest that root exudates of field crops can stimulate or inhibit nitrification (Subbarao *et al.*, unpublished results). We hope that future research efforts would be directed towards exploiting this remarkable plant attribute, which so far has not drawn much attention from plant scientists.

A. Evidence for Inhibition of Nitrification Based on Empirical Studies

In an early report Russell (1914) indicated field crops such as corn, wheat, sunflower, and sorghum could influence nitrification and nitrate formation in soils. As early as 1923, Lyon *et al.*, observed that fields planted with wheat and corn showed substantially lower soil NO_3^- levels compared to control plots without plants, fertilized in the same way. The NO_3^- concentrations ranged from 250 to 1913 mg kg^{-1} in the cropped field soils compared to 10,000 mg kg^{-1} in the plant-free control. Even after taking into account the total N recovered by crops, the quantities of NO_3^- formed in plots without crop were higher than fields planted with wheat or corn, suggesting that wheat and corn possibly had some inhibitory effect on soil nitrification. Subsequently, Moore and Waid (1971) showed that root washings of crops such as wheat, onion and ryegrass suppressed nitrification. Interference from microbial N immobilization or N losses from denitrification were ruled out in these studies. The low nitrification rates in soils incubated with root washings were attributed to nitrification-inhibiting factors/compounds released from wheat (*Triticum aestivum*) and ryegrass (*Lolium multiflorum*) roots (Moore and Waid, 1971). Similarly, the root exudates of several cultivars of sorghum (*Sorghum bicolor*) and sunflower (*Helianthus annuus*) showed the ability to inhibit nitrification in soils (Alsaadawi, 1988).

Genotypic differences in nitrification inhibition were observed for sorghum root exudates and tissue extracts (Alsaadawi *et al.*, 1986). Aqueous extracts of shoots and roots of sunflower (*Helianthus annuus* L.) were shown to inhibit nitrification in

soils (Alsaadawi, 1988). Barley plants had a stimulatory effect during the early growing season, but an inhibitory effect on nitrification from the onset of reproductive growth to maturity (Wheatley *et al.*, 1997). Considering the importance of nitrification in determining N recovery and utilization, it is rather surprising about how little we know about the role of crops in influencing nitrification.

B. Evidence for Stimulation of Nitrification Based on Empirical Studies

There is circumstantial evidence indicating that legumes stimulate nitrification. In a field study the influence of growing forage legumes (*Pueraria phaseoloides*, *Centrosema macrocarpum* and *Stylosanthes capitata*) on soil nitrification was compared with that of grass alone (*Brachiaria*) (*B. decumbens* and *B. humidicola*) (Sylvester-Bradley *et al.*, 1988). The nitrification rates were stimulated in soils where legumes were grown. There was no stimulation of nitrification in field soils planted with *B. decumbens*. In contrast, soils collected from *B. humidicola* pastures showed a substantial inhibitory effect on nitrification. In these studies microbial N immobilization was not observed (Sylvester-Bradley *et al.*, 1988). It is well established that legume roots release exudates that stimulate specific bacteria such as rhizobia for nodulation and nitrogen fixation. Nearly 10% of the total C fixed in some plants is released from their roots as root exudates (Bowen and Rovira, 1991; Grayston *et al.*, 1996). So, it is possible that the chemicals released from legume roots may specifically influence the nitrifier activity and soil nitrification.

Grain legumes do not invariably increase soil N levels because of their symbiotic nitrogen fixation. If the legume stover is removed from the field, the net effect of growing a grain legume crop on the N balance of the cropping system is mostly negative unless N contribution from fallen leaves, roots and nodules is considered (Giller, 2001). The net loss of N from the system by growing grain legumes can easily be 100 kg ha^{-1} or more, if estimates are solely based on above-ground plant parts. Soybean cultivation is one such example; its cultivation often leads to a significant exhaustion of mineral N in the soil when its stover is not returned to the soil. Soybean cultivation (i.e., replacing small grains cultivation with soybean) led to rapid increases in NO_3^- levels in water drained from the fields. This has been attributed to acceleration of SOM mineralization in soils (Aldrich, 1972). Preliminary research lends support to the hypothesis that root exudates from soybean indeed stimulate nitrifier activity and soil nitrification (Subbarao *et al.*, 2005).

It is likely that crops differ in their ability to influence nitrification in soils depending on the type of compounds, inhibitory or stimulatory released from roots through exudation. With some of the recent developments in detecting nitrifier activity using recombinant luminescent *Nitrosomonas*, it is possible to detect and characterize nitrification inhibitory activity in exudates from roots. The nitrification inhibition ability of major field crops

can be characterized as a first step, using some of the recently developed detection tools and assay protocols (Iizumi *et al.*, 1998; Subbarao *et al.*, 2005). This will open up possibilities from both the cropping system (i.e., utilizing crops that have inhibitory capability vs. stimulatory effect) and from the crop-improvement perspective, for improving N recovery, and minimizing N loss by exploiting their genetic potential to inhibit soil nitrification.

VIII. FUTURE OUTLOOK

The explosive expansion of human activity during the last two centuries through rapid industrialization and expansion of agricultural activities has resulted in massive changes in the N cycle of the planet. Currently N inputs into agricultural systems (i.e., industrially fixed N and N that is fixed from green manure legumes) have approached the level of N fixed by the natural systems of our planet. These large amounts of fixed N are added to agricultural systems that comprise only 11% of the Earth's surface (Newbould, 1989). Based on the projected population growth, and food demand, the N fertilizer inputs into agricultural systems need to double in the near future which would tend to further increase the amount of N lost to the environment. If production agriculture continues to move towards high-nitrification agricultural systems with the expansion and intensification of agricultural activities, there is potential for catastrophic consequences to our planet due to the destruction of the ozone layer, global warming and eutrophication. It is imperative that nitrification in agricultural systems be managed to minimize N leaks to the environment which are not only a serious economic and energy drain on society, but potentially also have long-term ecological and environmental consequences.

Major efforts so far in nitrification control have focused on agronomic management of N application using timing and adjustment of N inputs to minimize N residence time in soil. However, even this amount of management has not been widely or efficiently implemented. As a consequence, we are still losing more than 60% of the total N applied to agricultural systems which amounts to an annual economic loss equivalent of US\$ 17 billion, worldwide. The time is right for the development of N fertilizer products that resist nitrification by the development of a new generation of effective and affordable NIs. It is quite surprising to see that, although the chemical control of nitrification has been known since the 1960s, very little effort has been devoted towards the development of the next generation of nitrification inhibitors, especially ones that are efficient, cost-effective and suitable for both tropical and temperate production systems. As mentioned in Section VI, there are serious deficiencies associated with the currently available nitrification inhibitors. This has led to only limited adoption in farming systems. Considering the wide substrate range of AMO (an important enzyme involved in nitrification), it should be possible to identify a range of chemicals or chemical formulations that can be effectively deployed as additives to N fertilizers to control nitrification.

Mature natural ecosystems are in general not very leaky of N and tend to maintain tight control over N cycling, as N is often the most limiting factor for productivity in natural systems (Jordan *et al.*, 1979). We need to understand the specific biochemical mechanisms operating in these natural systems with high NUE where inhibition of nitrification has been established. Such genetic mechanisms, once identified and characterized, could be transferred to major field crops. Exploiting the natural mechanisms of nitrification-inhibition ability in major field crops should be one of the first research priorities for controlling nitrification in agricultural systems. Biological nitrification inhibition (BNI) in agricultural systems is a relatively new concept, which has the potential to revolutionize the efficiency of N uptake and utilization, and minimize N losses. Techniques are available to detect, quantify and characterize BNI ability of plants (Iizumi *et al.*, 1998; Subbarao *et al.*, 2005). Variability for BNI exists among plant species. This rather unique biological attribute needs to be genetically exploited using traditional, molecular and biotechnological tools to introduce this ability into major field crops. The first step towards this goal would be to develop crop varieties that have the capacity to affect nitrification through expression of BNI activity. Also, new approaches should be employed for developing smart N fertilizers that are resistant to nitrification. We hope that this review will stimulate research on the development of new approaches for regulating nitrification in agricultural systems for improved agricultural productivity and environmental quality. This area of research has not received the attention it deserves. Agricultural systems are presently much more open than natural systems. The suppression of nitrification in agro-ecosystems would be both cost-effective and environmentally-friendly.

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