The Effect of Nitrification Inhibitors on Nitrogen Cycle: A Comprehensive Review

Gao Dong*, Di Xiao, and Cai-Hua Zhang

1 China National Institute of Standardization, Room307, No. 4 Zhichun Road, Haidian District, Beijing, P.R. China
2 Department of Medicinal Chemistry and Molecular Engineering, College of Chemistry, Beijing Normal University, Room 803, No.19, Xinjiekouwai, Haidian District, Beijing, P.R. China
3 Southwest University of Political Science and Law, College of Politics and Public Administration, Chongqing, China

*E-mail: 202031150054@mail.bnu.edu.cn

Abstract. This paper analysed the nitrogen cycle and its current problem. On this basis, the article reviewed the history of nitrification inhibitors, then discussed and made conclusions about the effectiveness of NIs on N2O emission, nitrate leaching, yield increasing and NH3 volatilization.

1. The Nitrogen Cycle and Current Problem

Nitrogen is one of the primary nutrients important or the survival of all living organisms (Banerjee et al., 2002) [1]. The global nitrogen cycle is a process by which nitrogen of the atmosphere was brought into terrestrial and marine ecosystems through nitrogen fixation; the fixed nitrogen is then converted into different forms of nitrogen and return to the atmosphere as molecular nitrogen eventually. Naturally reactive nitrogen is created by biological nitrogen fixation, biomass burning, and lightening from N2 (Erisman et al., 2013) [2]. In terrestrial ecosystems, nitrogen is one of the major limitations of net primary productivity (LeBauer and Treseder, 2008) [3], and also commonly a limiting factor for the food production. Thus, more reactive nitrogen in agricultural systems is essential for providing enough food for the growing world population. Atmospheric nitrogen (78%) must be fixed and converted into some bioavailable form through natural or man-made processes. The anthropogenic N input is estimated to be only 3 Tg/yr 2000 years ago. Due to the invention of Haber-Bosch process in the early 20th century which could convert atmospheric nitrogen to ammonia, nitrogen input increased from 15 Tg /yr in 1860 to 156 Tg/yr in 1990, approximately 210 Tg/yr for now and is estimated to reach 267 Tg/yr in 2050 (Galloway et al., 2004; Galloway et al., 2013) [4,5]. On the other hand, the efficiency of N fertilizers is not very rich and not more than 50% of the applied N is absorbed by the crops, even in immense cash crop production, the rate reaches only 50–70% (Wiesler, 1998) [6]. Today, more and more N fertilizer is being used to sustain food security, however only 10% of the N fertilizer applied ends up in food, while 90% of N is lost (Galloway and Cowling, 2002) [7], which has drastic effects on environment; e.g. air pollution, greenhouse gas emission, water pollution in local, regional, and global scales (Galloway et al., 2004) [4]. By 2050, the human population will have probably increased by 2 to 4 billion in the world (Cohen, 2003) [8]. Since the human population is still rising, the human fixation of nitrogen continues to rise in order to satisfy the basic human need, however, the negative effects on ecosystems and human-
beings are becoming more and more serious and may eventually offset the whole benefits. The consequences of nitrogen loss are numerous. One of them is N2O problem. N2O plays an important role in environmental terms due to their global warming potential. Although the concentration of N2O is considerably lower than that of CO2, account it could cause greater impact on the greenhouse effect than CO2. Furthermore, N2O not only causes the global warming but also damages ozone layer (Ravishankara et al., 2009) [9]. Currently, food production is thought to be the largest anthropogenic source of N2O, accounting for about 60% of the total emissions (Syakila and Kroeze, 2011) [10]. Nitrate leaching has also increased significantly in the past decades (Bouwman et al., 2002) [11], which had led to serious environmental problems like groundwater pollution with nitrate. Some studies have shown that non-Hodgkin lymphoma and colorectal cancer has a connection with nitrate in drinking water, (Gulis et al., 2002) [12]. Nitrogen loss has also caused serious problem of acidification and eutrophication in aquatic ecosystem and direct foliar damage can also be caused by the N deposition. N deposition could also reduce terrestrial plant diversity in a range of ecosystem (Erisman et al., 2013) [2].

2. History of Nitrification Inhibitors

Although there are several important compounds, such as mineralization, ammonification and denitrification in terrestrial N cycle. The role of each varies a lot at different ecosystem. In the typical agricultural system, nitrification is the dominant process (Subbarao et al., 2013b) [13]. The biological oxidation of ammonium (NH4+) or ammonia (NH3) via nitrite (NO2-) to nitrate (NO3-) is called nitrification. Nitrification process was discovered in 1877 by the German workers Schloesing and Muntz (Payne, 1986) [14]. The concept of controlled nitrification is to make nature mineralization produce NH4+ and inhibit the first step of nitrification so that N is preserved in the root zone, as NH4+ is more difficult to loss from soil (Amberger, 1989) [15]. In recent years, all kinds of compounds have been identified and used as nitrification inhibitors, particularly in agricultural soils. Nitrification inhibitors are compounds, it could dominate the nitrosomas bacteria which activities have significant connection with the bacterial oxidation of NH4+ to NO2– in the soil. (Zerulla et al., 2001) [16]. It first came out with the invention of N-serve (trade name for nitrapyrin of Dow chemical Company) in about 1960s (Prasad and Power, 1995; Slagen and Kerkhoff, 1984) [17, 18]. Since 1960, numerous compound and products have come into use to control nitrification in soil, but many of them haven’t been widely tested, ie: Nitrapyrin (2-Chlor-6-(trichlormethyl) pyridine), AM (2-mino-4-chloro-6-(tri-chloromethyl) pyridine), DCD (Dicyandiamide), ST (2-sulfanil-amido thiazole), TU (thiourea), TERRAZOLE (5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole), MBT (mercaptobenzothiazole), C2H2 (acetylene) (Prasad and Power, 1995) and DMPP (3,4-dimethylpyrazole phosphate) [17]. Nowadays, two nitrification inhibitors including DCD and DMPP has been frequently researched and repeated in the literature. For example a search in web of science with key word nitrification inhibitor, year: 2000-2013, in 150 result of all, 95 papers were about DCD, 45 papers were about DMPP, 9 papers were about Nitrapyrin, and only less than 10 papers were about other inhibitors.

The biochemical mechanisms of various nitrification inhibitors that affect nitrification process varies significantly (Prasad and Power, 1995) [17]. It has been shown that NH3 monoxygenase (AMO) may account for the inhibitory effects of many compound. Since the competition for the active site and and a large class of compounds containing thiono-S inhibit AMO activity by binding with Cu within the active site is the inhibitory effects of many compounds (McCarty, 1999) [19]. The mechanism for nitrification inhibitor of heterocyclic N compound is still unknown, existing researches show that the ring N in the soil may be influenced by the nitrification inhibitor. (McCarty, 1999) [19]. A comparison of the effect of various unsubstituted heterocyclic N compounds on the oxidation of NH3 in soil revealed that substances which is contained by two or three adjacent N atoms significantly inhibit nitrification, while those having two or three nonadjacent ring N atoms have little influence on ammonia oxidation (McCarty and Bremner, 1989) [20]. As a foundation to understand the mechanisms of nitrification inhibitors, the understanding of nitrification process has been improved in recent years. Along with Nitrosomonas spp, Chemolithoautotrophic ammonia-oxidizing bacteria (AOB) was regarded as the major responsibility for the rate –
limiting steps of nitrification (Kowalchuk and Stephen, 2001) [21]. However, in 2004, ammonia-oxidizing archaea (AOA) began to play a crucial part in the nitrification process in various soils (Konneke et al., 2005; Treusch et al., 2005; Venter et al., 2004) [22-24].

3. Effectiveness of Nis on N2O Emission, Nitrate Leaching, Increasing Yield and NH3 Volatilization

A large number of studies in recently 10 years have demonstrated evidence that the addition of DCD could significantly reduce the N2O emission. Cui et al. (2011) [25] in the intensive vegetable production system field experiment found that N2O emissions factor was reduced by 72.7–83.8% with DCD. Di et al. (2007) [26] in a grassland system experiment reported that DCD could effectively reduce N2O emissions in all 4 different kinds of soils with an average of 70% reduction. Qiu et al. (2010) [27] found that in the perennial pasture system DCD decreased 40% N2O emission in summer and 69% in winter. Liu et al. (2013) [28] demonstrated that the DCD treatment significantly decreased by 35% of the N2O annual emission through test in wheat-maize cropping system for the whole year.

Several studies have demonstrated that DMPP could also significantly reduce nitrous oxide emissions in ranges of ecosystem. In a 3-year cropping system experiment DMPP decreased by 49% of N2O release averagely (Weiske et al., 2001) [29]. Results from experiment conducted in China showed that DMPP decreased by 38% the annual N2O emission in a wheat-maize cropping system (Liu et al., 2013) [28]. In a grassland system in England DMPP reduced 32% of N2O release (Dittert et al., 2001) [30], while it decreased remarkably N2O emissions in a vegetable production system in Germany during the cropping season and winter period, resulting in annual 45%, 40% N2O reduction from 2 years experiment (Pfab et al., 2012) [31]. Menendez et al. (2006b) found 29% reduction in N2O emissions when adding DMPP to slurry, but not when adding DMPP to ammonium sulfate nitrate ASN in grassland system [32]. Macadam et al. (2003) found that DMPP reduced N2O emissions for both cattle slurry (61% reduction) and mineral fertilizer (57%) [33].

On the other hand, the effect of NI on nitrate leaching is controversial. Results from a 3 years field experiments in pasture system (Monaghan et al., 2013) showed that single mid-winter applications of DCD had no significant effects on reducing N leaching losses from the forage crop [34]. While another 2 growing seasons field experiment in an irrigated maize crop under Mediterranean conditions (Diez et al., 2010) showed that the usage of DCD decreased soil by 30% NO3-concentration and achieved significant reductions in nitrate leaching [35]. Díez-López et al. (2008) found that the utilization of DMPP with urea reduced nitrate leaching in an irrigated maize crop in two growing seasons under Mediterranean conditions [36]. In a study (Yu et al., 2007), a multi-layer soil column device was used and the results showed that within 60 d of experiment, the use of DMPP could decline the concentrations of nitrate and nitrite [37]. And another finding was that there was no significant difference among the nitrate, ammonium and nitrite concentrations in the DMPP treatments in the soil solution under 40 cm depths of soil profile with the increasing nitrogen application level. No large difference on ammonium concentrations in soil solution collected from deep profile under 20 cm between treatment with DMPP and treatment without DMPP. While, on the contrary, Wu et al. (2007) in soil column experiment studies found that in sandy loam soil and clay loam soil DMPP decreased NO3-leaching but ammonium leaching increased slightly [38], even though, the total N leached was less than that in the DMPP treatment than without DMPP treatment.

There have been also controversial results when considered the yield effects of NI. A study in intensive vegetable production system with three kinds of vegetables and two different soils showed that DCD significantly increased the yields of capsicum and radish in alfisol soil, but decreased amaranth yield in fluvisol soil, while others vegetable yield were not affected (Cui et al., 2011) [25]. Liu et al. (2013) reported that in a one year field experiment in winter wheat and summer maize system the use of DCD increased 8.5% annual yield [28]. The results from a 3-years 136 field trials which conducted under various soil-climatic conditions in western and southern Europe (Pasda et al., 2001) showed that DMPP increased yield of grain (winter wheat, wetland rice, grain maize ), yield of tuber (potatoes) [39].
corrected sugar yield (sugar beets), and biomass (carrots, lambs’ lettuce, onions, radish, lettuce, cauliflower, leek, celeriac). Fangueiro et al. (2009) [40] reported that on annual Ryegrass dry matter yield of 8698 kg/ha was obtained with the DMPP applied at the 2 kg/ha rate against 4767 kg/ha yield of slurry only treatment. A 9% annual crop yield increasing was obtained in a winter wheat summer maize system in China (Liu et al., 2013) [28]. However, a meta-analysis with 144 observations of recent studies in Germany showed that nitrogen fertilizers with NIs didn’t significantly affect the yields of all investigated crops (Hu et al., 2014) [41]. A cut plot experiment which was made at two sites in Ireland showed that application of DCD increased herbage production about 15% in at one site in 1 year while no effect at the other site (O’Connor et al., 2013) [42]. Figure 1 shows the comparisons of N2O, yield, leaching effects of NI can be seen below (Figure 1).

![Figure 1](attachment:image.png)

**Figure 1.** Comparison of yield increases, leaching decreases, N2O emission decreases between DCD, DMPP and all NIs, and comparison of N2O decreases between different system of crop and pasture.

As nitrification inhibitor keeps nitrogen in the form of NH4+ instead of NO3- for a longer time in soil, it could also cause the increase of NH3 volatilization. There are some argues about the effects of NI on NH3 volatilization. Blaise et al. (1997) reported that NH3 loss was about 9% more with DCD and urea than with urea alone in a laboratory incubation study [43]. In another volatilization chambers experiment, the addition of DCD caused a 5-16% increase in NH3 volatilization losses of the fertilizer N applied as Urea (Soares et al., 2012) [44]. In a rice-wheat cropping system, application of DCD increased NH3 volatilization in wheat by 7%, however it had no effect with fertilizer in rice as urea (Banerjee et al., 2002) [1]. With a meta-analysis of 46 data set from 21 studies from 1970 to 2010 (Kim et al., 2012) suggested that NH3 volatilization significantly increases with NI application [45]. However, the NI used in most of these experiments was DCD. In one study compared DCD and DMPP (Wissemeier et al., 2002) reported that in the ASN/DCD treatment, the NH3 volatilization was slightly higher (18.0 kg/ha) than control (15.4
kg/ha) [46], while the NH3 volatilization of treatment with ASN/DMPP treatment was even lower than control (14.2 kg/ha). In a field experiment with cut grassland system, no significant NH3 volatilization increase was found with DMPP application when applied together with slurry (Menendez et al., 2006a) [47]. Another field experiment (Li et al., 2009) in a farmland with crop rotations also showed that the application of DMPP had no significant effects on NH3 volatilization loss fluxes [48].

Nitrification inhibitors have been researched for over 5 decades, and its significant effects on the reducing of N2O emissions have been tested and proved by various researches. However, the underlying mechanisms is still insufficiently discussed, even under well-controlled laboratory conditions. Nevertheless, further study either laboratory or field research still needs to be conducted to recognize the contribution of nitrifier denitrification.

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