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Nitrogen Transformations Associated with N₂O Emissions in Agricultural Soils

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<http://dx.doi.org/10.5772/intechopen.71922>

Abstract

Nitrogen (N) is one of the most important plant nutrient, and its availability and transformations are vital for net primary production. Soil N transformations include mineralization, nitrification and denitrification processes. Nitrogen mineralization transforms organic N into inorganic N, providing available N for crops. Both nitrification and denitrification are microbe-driven processes associated with nitrous oxide (N₂O) emissions. N₂O emissions from agricultural soils decrease N fertilization efficiency and potentially induce global warming. The mitigation of soil N₂O emissions in agricultural practice is essential for sustainable development of agriculture considering the environmental effect of N₂O. Various strategies have been proposed for the mitigation of N₂O emissions. Nitrification inhibitors have been demonstrated to be useful in decreasing soil N₂O emissions, including the application of nitrification inhibitors, such as dicyandiamide (DCD) and 3,4-dimethylpyrazole phosphate (DMPP). Recently, biological nitrification inhibitors have also attracted researchers' attention, which may be more environment-friendly. In addition, biochar commonly used as soil ameliorant to improve soil quality and C sequestration could also mitigate soil N₂O emissions. Once all effective strategies would be widely implemented, more environment-friendly agriculture could be expected.

Keywords: fertilizer efficiency, global change, mitigation, nitrogen emissions, nutrition

1. Introduction

Nitrogen (N) is one of the most important elements for plant growth and hence it is vital for ecosystem's primary production [1]. In the background of global climate change, understanding soil N balance in agricultural soils is important for sustaining the development of modern agriculture and mitigation of climate change considering the substantial contribution made by agriculture to climate change [2–4].

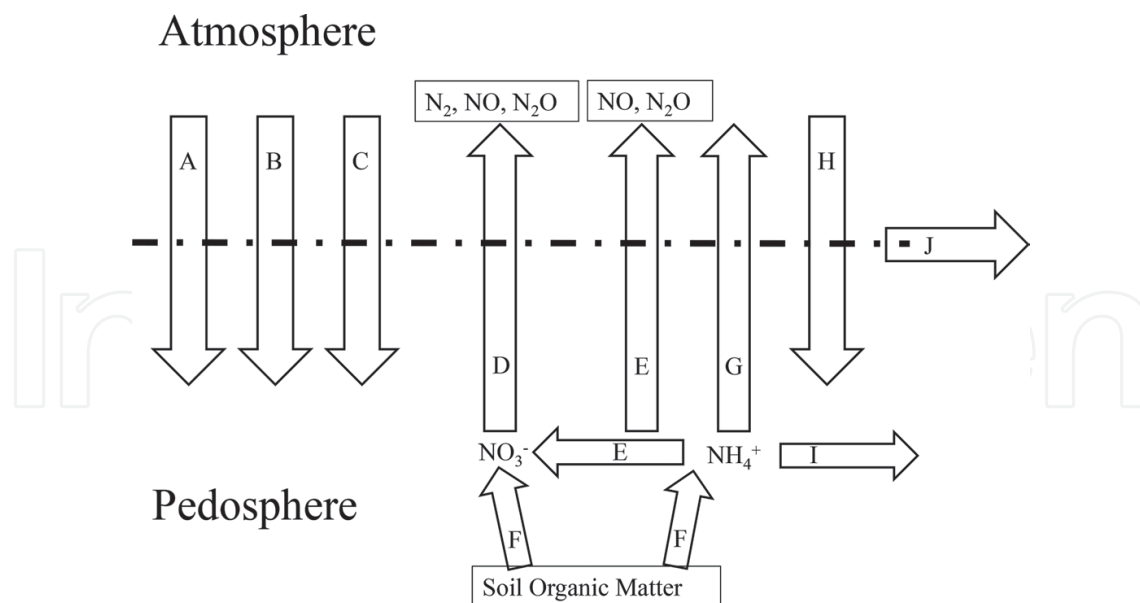


Figure 1. Simple diagram showing N cycle between atmosphere and agricultural soil. Note: (A) N deposition; (B) N fixation; (C) fertilization; (D) denitrification; (E) nitrification; (F) organic N mineralization; (G) plant uptake; (H) litter decomposition; (I) soil immobilization; (J) N volatilization, leaching and crops harvest.

In general, soil N mainly originates from atmospheric N deposition (both dry and wet deposition) (**Figure 1**) and biological fixation in natural ecosystems [5]. In agricultural soils, however, N input via anthropogenic activities (e.g., fertilization practice) has been considered as the most important way [6]. Fertilization in agricultural practice could introduce many kinds of N into soil ecosystems. For example, both organic and inorganic N, in liquid or particle status, could often be observed in agricultural fertilization practice. In addition to N input by anthropogenic activities, N output via crops harvest or human-induced runoffs with irrigation could occur simultaneously in agricultural soils (**Figure 1**).

Moreover, the complicated N transformations going on in soil ecosystems also account for large part of N output from soils [7]. For example, N mineralization associated with transforming organic N into inorganic N might induce more N leaching with runoffs or underground water; nitrification process could increase N loss from soils via gas emissions [2, 7, 8] (**Figure 1**).

Thereby, understanding the process and mechanisms underlining N transformations in agricultural soils would be vital for more efficient fertilization practice and agriculture management.

2. Nitrogen transformations in agricultural soils

2.1. Overview of soil N transformations

Except for N transformation process ongoing during litter decomposition process at the litter-soil interface, N was also transformed by processes including mineralization, nitrification, denitrification, and so on, in agricultural soils [9].

While organic N accounts for larger part of total soil N, only soil inorganic N (mainly including ammonium and nitrate N) derived from mineralization of organic N is readily available for plant. Thereby, organic N mineralization is an important factor controlling levels of soil inorganic N availability, which is vital for crop production. In agricultural soils, most crop residuals were harvested by agricultural activities, while some of them were returned into soils as roots, rhizospheric deposition or aboveground litter. During decomposition of these organic matters, most inorganic N would be released into surrounding soils via mineralization of organic N. In these processes, N uptake by plant from soils was returned into soils again, during N mineralization process.

Nitrogen produced by mineralization process, including both ammonium and nitrate N, was readily available for crop growth. If there was no leaching or loss via runoffs, all available N will be adsorbed by plant roots and used for growth. However, nitrification and denitrification processes (both produce greenhouse gas N₂O) also occurred between ammonium and nitrate N, causing new balance among different N components [2, 4, 7].

In addition, soil ammonium N could also be immobilized by soil particles, and both important inorganic N may loss with surface runoff, further decreasing its availability level. Before mineralized by microbes, soluble organic N was also reliable to be removed by runoffs.

2.2. General methods in studying soil N transformations

2.2.1. Net and gross N transformation rates

Due to complicated interactions among all soil N transformation process, net N transformation rates have been widely studied [10, 11]. Once plant roots were excluded, changes in soil N levels could be considered as results of transformations. Specifically, when soil mineralization rates were examined, both ammonium and nitrate concentrations were determined both at the beginning and at the end of the study. Net N mineralization rates were considered as changes in concentration of both inorganic N per given time unit [11, 12]. The equations used for the calculation were as follows:

Net N mineralization rate:

$$N_{\text{mineralized}} = \frac{[(\text{Nitrate}_f + \text{Ammonium}_f) - (\text{Nitrate}_0 + \text{Ammonium}_0)]}{T_{\text{days}}} \quad (1)$$

where $N_{\text{mineralized}}$ = net N mineralization rate, expressed as mg N kg⁻¹ soil day⁻¹, Ammonium_f = final ammonium concentration, expressed as mg NH₄⁺-N kg⁻¹ soil, Ammonium₀ = initial ammonium concentration, as mg NH₄⁺-N kg⁻¹ soil, T_{days} = incubation time, days.

Similarly, net nitrification rates and ammonification rates were calculated as follows:

Net nitrification rate:

$$N_{\text{nitrified}} = \frac{(\text{Nitrate}_f - \text{Nitrate}_0)}{T_{\text{days}}}, \quad (2)$$

Net ammonification rate:

$$N_{\text{ammonification}} = \frac{(\text{Ammonium}_f - \text{Ammonium}_0)}{T_{\text{days}}}, \quad (3)$$

where $N_{\text{nitrified}}$ = net nitrification rate, expressed as $\text{mg NO}_3^- \text{-N kg}^{-1} \text{ soil day}^{-1}$, Nitrate_f = final nitrate concentration, expressed as $\text{mg NO}_3^- \text{-N kg}^{-1} \text{ soil}$, Nitrate_0 = initial nitrate concentration, as $\text{mg NO}_3^- \text{-N kg}^{-1} \text{ soil}$, Ammonium_f = final ammonium concentration, expressed as $\text{mg NH}_4^+ \text{-N kg}^{-1} \text{ soil}$, Ammonium_0 = initial ammonium concentration, as $\text{mg NH}_4^+ \text{-N kg}^{-1} \text{ soil}$, T_{days} = incubation time, days.

Meanwhile, with the development of isotope labeling strategy, more studies have been conducted to determine the gross N transformation rates in agriculture and forestry soils [13–16]. By labeling N in ammonium or nitrate N, N element could be traced during the complicated transformation process. Thereby, the gross N transformation rates could be obtained using the isotope labeling method.

2.2.2. *In situ and ex situ studies on N transformations*

Studies on N transformations could also be sorted by study place or site, into *in situ* or *ex situ* studies. Laboratory soil incubation studies were widely used to examine N transformations in environmental science [10, 17]. During laboratory incubation, the environmental factors could be easily altered to check their role played on N transformation rates. Thereby, studies conducted in incubators are more operable than those *in situ*. For example, incubation studies could be manipulated with different soil water content, incubated temperature, or even aeration status. However, not all N transformation studies could be conducted in the laboratory. In agricultural ecosystems, the dynamics of soil N might be important for fertilization practice in field crops research and management. In this condition, laboratory incubation studies are no longer applied. Instead, *in situ* soil core incubation is more suitable. Similar to laboratory incubations, both concentrations of soil inorganic N at the beginning and at the monitored date should be determined of the soil column. Moreover, the soil column incubated *in situ* for study should be isolated from the surrounding soils to prevent potential N uptake by roots of crops.

In recent years, ion-exchange resins (Unibest PST-1, Unibest, Bozeman, MT, USA) have been widely used for *in situ* studies on soil N availability [18, 19]. In these studies, incubated soils cores were isolated from surrounding soil. Exchangeable anion/cation resin was used to capture any inorganic N moved into or out of the incubated soil core [19]. Changes in soil inorganic N in soil core and the resin relative to that of the initial soil core with time were considered as net N mineralization rate. Similarly, changes in soil nitrate N with time were considered as net nitrification rate.

3. Nitrogen transformations associated with N_2O emissions

3.1. Illustration of N_2O emissions

3.1.1. Soil N_2O production

N_2O is one important component of greenhouse gas emitted from soil. The global warming potential of N_2O is much larger than that of methane and carbon dioxide (CH_4 and CO_2). According to the latest report, even though not so much as CH_4 and CO_2 in atmospheric environment, the

global warming potential of N₂O is 265 times that of CO₂, while CH₄ is only 28 times [3]. Thereby, N₂O might have contributed substantially to global warming considering its larger global warming potential [3, 8]. Importantly, most of the atmospheric N₂O was emitted from soils, especially those fertilized by N fertilization in agricultural ecosystems. Hence, studying the way N₂O is produced, emitted and adsorbed in agricultural soils would be important for mitigation of soil N₂O emissions and hence for mitigation of atmospheric N₂O in this climate changing world.

Soil N₂O has been demonstrated to be the by-product or production of N transformation process by previous studies [20]. Specifically, the main process producing N₂O in soil ecosystems, namely nitrification and denitrification process, transformed ammonium N to nitrate N, and nitrate N to N₂, respectively [21, 22]. The processes associated with N₂O production could be described by hole in the pipe model (HIP, **Figure 2**) [20]. The model showed that N₂O was produced by complete nitrification process and was produced during denitrification process. In both processes, nitric oxide (NO) was also produced by both nitrification and denitrification processes, which was also considered as an important GHG [23–25]. Recently, in addition to N₂O, NO has also attracted increasing attention due to their role played in altering atmospheric chemistry and global climate [26–29].

3.1.2. Measurement of soil N₂O emissions

Methods being developed for the measurement of soil N₂O emissions rate including two different sets. This first one was used to measure soil N₂O emission rate in laboratory incubation studies. Different to that conducted *in situ*, incubation studies monitored soil N₂O emission based on soil mass rather than soil surface area. Thereby, those studies measured N₂O emission rate based on soil mass per time. Equation developed for the calculation of N₂O emission rate of incubated soil could be described as follows [17, 30, 31]:

$$E = P \times V \times \frac{dc}{dt} \times \frac{1}{RT} \times M \times \frac{1}{m} \times \frac{1}{t} \quad (4)$$

where E refers to emission rates of soil N₂O (ng g⁻¹ h⁻¹), P is standard atmospheric pressure (Pa), V is headspace volume of the incubation flask (cm³), c is the concentration of N₂O (ppb),

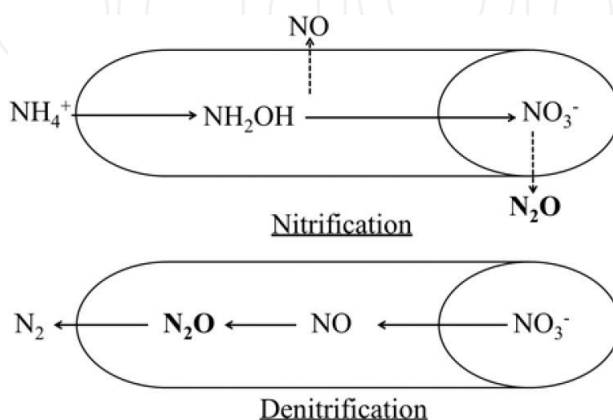


Figure 2. Simple diagram showing the production process of soil N₂O.

t is the time between two sample collections (h), R is the universal gas constant, T is the absolute air temperature (K), M is the molecular mass of N_2O ($g\ mol^{-1}$), and m is incubated soil mass by dry weight basis (g).

Relative to laboratory incubation studies, *in situ* studies could capture effects of various factors on soil N_2O emissions in natural environment. These studies generally calculated soil N_2O emission rate based on soil surface area. In general, *in situ* measurements could be conducted using static opaque chamber/gas chromatography method. To be specific, circular or square grooved collars should be buried into soil, with groove filled with water to seal the gas collection chamber [32, 33]. When soil N_2O emission rate would be measured, open-bottom cylindrical or cubic PVC gas sampling chamber would be fit into the groove. To exclude the potential effects of temperature variation during gas collection process, gas chambers were usually wrapped by foam and aluminum foil. Inside each chamber, battery-powered fans were used during gas accumulation process to mix air samples. Gas samples were usually collected manually or automatically using single-use syringes or sir bags, respectively. After gas collection, gas chromatograph with electron capture detector was used to measure N_2O concentrations. Soil N_2O emission rates were usually determined by the equation as follows [31–33]:

$$F = P \times V \times \frac{dN_2O}{dt} \times \frac{1}{RT} \times M \times \frac{1}{A} \times \frac{M_n}{M} \quad (5)$$

where F refers to soil N_2O emission rates ($mg\ N_2O\ m^{-2}\ h^{-1}$), P is the standard atmospheric pressure (Pa), V and A are the volume (m^3) and interior bottom area (m^2) of the gas collection chamber, R stands for universal gas constant, T is the absolute air temperature (K) when the gas sample was aspirated and M_n and M are the molecular masses of N and N_2O ($g\ mol^{-1}$), respectively.

When cumulative emissions were needed for study purpose, total soil N_2O emissions within a given time could be obtained by multiplying average soil N_2O emission rate and the corresponding time span [17, 33].

It should be noted that both methods were used to obtain the net soil N_2O emission rates. During measurement, soil might be source or sink of N_2O depends on soils used for studies. However, when results were positive based on two equations, it could be determined that soils were emitting N_2O . Similarly, when values were negative, soil could be adsorbing N_2O in the corresponding studies.

3.2. Factors impacting soil N_2O productions

3.2.1. General factors impacting soil N_2O productions

According to the model shown in **Figure 2**, factors impacting nitrification and denitrification could also be able to influence the production and emission of soil N_2O . As have been reported by previous studies, factors impacting nitrification process including quantity and quality of soil N input, soil moisture (water holding capacity) [34, 35], soil temperature [10, 17, 30], irrigation and tillage practices, soil type, soil oxygen concentration, dissolved organic C availability (controlling substrate availability of soil microbes) [17, 36], additives for soil

amelioration, vegetation or crop types [37], land use change and soil pH [36, 38]. While soil N provided substrate for transformation process producing soil N₂O, other factors regulated N₂O production process mainly via indirect effects on soil microbial activities.

For example, soil temperature is the key factor controlling microbial activities. Since both nitrification and denitrification processes have been demonstrated as being driven by nitrification and denitrification bacteria, soil temperature could impact both transformation processes via its effects on bacterial activities. According to previous studies, nitrification process preferred temperature between 25 and 35°C, while it will be inhibited when soil temperature decreased below 5°C or increased above 50°C [39]. Moreover, the favorable temperature for denitrification falls within 30 and 67°C [40].

In addition, soil acidification levels as shown by soil pH are also important for microbial activities. To be specific, the ratio of N₂O in denitrification process would increase with relatively lower soil pH, indicating enhancement of denitrification bacteria activities. Similarly, activities of some nitrification bacteria would also increase in response to lower pH.

Soil aeration also controls the quantity of N₂O by nitrification or denitrification process. In well-ventilated soil environment, nitrification process could be complete while denitrification process posed at stage producing N₂O. Since complete nitrification process was also accompanied by N₂O production, under the same environmental conditions, more N₂O would be produced in upland soils relative to flooded soils.

3.2.2. *Factors impacting N₂O production in agricultural soils*

In agricultural soils with intensive anthropogenic disturbance, soil N₂O productions became more complicated compared with those in natural soils [4]. Agricultural practice generally including fertilization, tillage, water regime [34], and so on, all of which could alter soil physical and chemical properties, impacting soil N₂O productions [4].

First, agricultural soils received much more N input via fertilization, increasing N availabilities for nitrification and denitrification process [2, 4, 8]. Except for increasing N availability directly, fertilization types, quantity of fertilizations, fertilization method and the time when soil was fertilized together regulated N transformation process. In general, N fertilization including inorganic N or organic N input in agricultural management. In organic agriculture, activities of denitrification bacteria were higher, potentially facilitating the denitrification process, decreasing soil N₂O productions [41]. Indeed, soil N₂O emissions were found lower in organic agriculture than conventional agriculture in another study by Phillips [42].

Second, farmland with decreased-tillage or non-tillage management potentially enhance the accumulation of soil organic C [43]. Due to the balance between C and N regulated by C to N ratio, increased soil organic C might be accompanied by increased fixation of soil N and hence less N₂O emissions from agricultural field [36]. However, increased soil N₂O emissions were also observed in studies on decreased tillage farmland [34, 44, 45]. Moreover, soil tillage could also impact soil aeration conditions and indirectly regulate soil N₂O emissions via effects on microbial activities. Thereby, soil tillage and other disturbance management may be important in impacting soil N₂O emissions [34, 45, 46].

Water regime, especially that in paddy field, plays an important role in controlling soil N_2O production and emissions [47–49]. When paddy field was flooded, soil aeration was inhibited, anaerobic microenvironment was hence cultivated. In this kind of condition, N_2 became the main production of denitrification process, soil could be considered as the sink of N_2O , as almost no emissions were observed. Meanwhile, flooded soil was generally favorable to methanogens, which were associated with soil CH_4 production. Indeed, flooded soil, generally in paddy field, has been demonstrated to be the main source of CH_4 [47]. However, during drainage time, paddy field was not flooded any more, especially during time when soils were humid (i.e., wetting and drying cycles), aerobiotic soil environment was formed, and soil N_2O emission rate could reach a peak [31]. In this kind of environment, soil is not completely dry but experiencing wetting and drying cycles, allowing more oxygen in soil pores, increasing the production of N_2O [50]. Yan et al. [51] studied the correlations between soil water content and soil N_2O emissions, reporting the largest soil N_2O emission rate when soil water content was equivalent to water holding capacity. Thereby, soil water content and soil aeration condition interact in impacting soil N_2O emissions in agricultural soil.

3.3. Mitigation of N_2O emissions from agricultural soils

Various strategies have been developed for the mitigation of soil N_2O emissions, especially in agricultural ecosystems. Agricultural management including fertilization, tillage, crop rotations, and so on has been employed in mitigation of soil GHG, especially N_2O emissions. Not only because N_2O induce global warming, but also N losses that accompanied the production and emission process of N_2O . To increase fertilization efficiency, the economy of agriculture management, and the benefit for environment, more efficient mitigation strategies are still needed. Presently, nitrification inhibitor has been widely used in agricultural management and has been demonstrated to be much more effective.

Nitrification inhibitor interrupted the transformation process from ammonium N to nitrate N, which could decrease N losses from soil (emissions or leaching, for example, Marsden et al. [52]) and increase N availability level for crops and hence the adsorption of ammonium N. Using nitrification inhibitors could potentially decrease soil emissions via interruptions on both nitrification and denitrification processes simultaneously. Chemical nitrification inhibitor and biological nitrification inhibitor are two important choices in recent studies.

3.3.1. Chemical nitrification inhibitors

Chemical nitrification inhibitors are human-synthesized materials. By decreasing soil N loss induced by nitrification and denitrification process, nitrification inhibitors could enhance efficiency of N fertilization. Thereby, nitrification inhibitors were also used as additives for N fertilizers. Nitrogen fertilizers with these additives (or similar additives like urease inhibitors, etc.) were usually used and called as enhanced efficiency nitrogen fertilizers [53].

Dicyandiamide (DCD) and 3,4-dimethylpyrazole phosphate (DMPP) are two most widely used nitrification inhibitors [54]. Both inhibitors interrupted the oxidation of ammonium N, limiting the important step occurred in nitrification process. Even though both inhibitors could

be used with N fertilization and were effective in mitigation of soil N₂O emissions, DMPP has been demonstrated to be less phytotoxic and used at lower rate relative to DCD in general. The efficacy of both inhibitors in mitigation of soil N₂O emissions could depend on temperature, soil chemical and physical characteristics, and so on. However, the difference in mitigation efficacy could also have been induced by the mobility of inhibitors in soil environment.

However, it should be noted that attentions should be paid to the negative effect on soil N availability [55] or food security [54] induced by applications of nitrification inhibitors. The first negative effect is the potentially increased ammonia (NH₃) volatilization induced by nitrification inhibitors [55]. Indeed, nitrification inhibitors decreased the rate of nitrification and denitrification process, potentially prolonged the retention time of ammonium N in soil environment, increasing the possibilities of more ammonia volatilization. Increased ammonia emissions would on one hand decrease the efficacy of N fertilization practice, and importantly, on the other hand, have economic and environmental consequences [53] considering their potential driving effect on soil N₂O emissions.

To reduce potentially increased ammonia volatilization after application of nitrification inhibitors, manufactures developed urease inhibitor N-(n-butyl) thiophosphoric triamide (NBPT) [56, 57]. Application of NBPT has been proved effective in reducing pasture soil urease activity and mitigating ammonia volatilization [58, 59]. The combination of nitrification inhibitor and NBPT could also decrease the yield-scaled N₂O emissions relative to treatments only with fertilizer in banana plantations [56].

The second caution is the grain yield and quality following alteration in soil N components [54]. While it is rational to expect that grain N concentration may increase in response to soil with longer N retention time, there are studies demonstrating no such effect in grain N following DMPP application [54]. A recent study on banana plantations in tropical China reported decreased yield-scaled N₂O emission, but banana yield showed no significant difference between N fertilization treatment and N with inhibitors [56]. Thereby, the efficiency of N fertilization practice with nitrification inhibitors might be crops or vegetation-type dependent, which should be considered in future applications.

There are also cautions on N fertilizer types that could be used combining with the application of nitrification inhibitors. To maintain the efficiency of nitrification inhibitors, only several N fertilizers could be widely used with nitrification inhibitors. Due to higher cost of theses fertilizers, wide adoption in agricultural practice became more difficult [59]. Recently, new compounds with higher nitrification inhibitor efficiencies have been developed, such as 3,4-dimethylpyrazole succinic (DMPSA), which is more stable when applied combined with other basic fertilizers (e.g., calcium ammonium nitrate) at basic conditions [54, 59].

3.3.2. *Biological nitrification inhibitors*

In tropical grassland and forest ecosystems, nitrification rate was found much slower relative to that in other similar soils. In further studies, it was found that some plant species synthesized important organic compounds and released these compounds into surrounding soils via roots [60]. These compounds were found being able to inhibit nitrification process, imposing similar

effects on nitrification process as chemical nitrification inhibitors. They were called biological nitrification inhibitors as they were not human synthesized. Thereby, biological nitrification inhibitors are organic materials that have similar negative effects on nitrification process exuded by plant.

Biological nitrification inhibitors generally including phenolic compounds, alkaloid, isothiocyanate and terpenoid [60, 61]. For example, chemicals produced by *Arbutus unedo*, including phenolic compounds galocatechin and catechin were able to decrease soil N_2O emissions [62]. In tropical grassland of Africa, both *Brachiaria humidicola* and *Brachiaria decumbens* were found be able to release biological nitrification inhibitors by producing linoleic, and so on, which enable them to survive in the low-N south Africa Savannas [63].

Compared with chemical nitrification inhibitors, biological inhibitors were environment-friendly in their producing process and application area. However, due to varying ability in synthesizing these compounds, further studies in how to cultivate species with stronger ability in producing these compounds are still needed.

3.3.3. Other gradients incorporated into agricultural soils

Other efforts have also been tried in decreasing soil N_2O emissions. In agricultural and forest soils, biochar has been used to improve soil quality and C sequestration [64–67]. Biochar is produced by slow pyrolysis of crop residues, household garbage, poultry litter, wood chips, or some other similar materials at high temperature (pyrolysis temperature generally between 400 and 600°C) without oxygen [67]. The physical characteristics of biochar enable it to be an ideal soil ameliorant. By applying biochar, soil aeration could be improved significantly, providing more oxygen and hence enhancing soil microbial activities. Due to its special physical characteristics, biochar could also prevent soil N leaching by adsorbing nitrate N (temporary immobilization, which would not impose negative effect on plant nutrient availability forever).

Acknowledgements

We acknowledge funding support by National Natural Science Foundation of China (Award number: 41501317), China Postdoctoral Science Foundation (Award number: 2017M612153), and Key Project of Jiangxi Education Department (Award number: GJJ160348).

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