



Fingerprint natural soil N₂O emission from nitrification and denitrification by dual isotopes (¹⁵N and ¹⁸O) and site preferences



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ARTICLE INFO

Article history:

Received 18 December 2015

Received in revised form 25 May 2016

Accepted 26 May 2016

Keywords:

N₂O

Nitrification

Denitrification

$\delta^{15}\text{N}_2\text{O}^{\text{bulk}}$

$\delta^{18}\text{O}$

Site preference

ABSTRACT

Nitrification and denitrification are the main contributors to soil N₂O production and emission, and distinguishing their contributions to N₂O emission under natural conditions is vital to deciphering nitrogen biogeochemistry. Stable isotopes have provided insight to resolve this problem, of which $\delta^{15}\text{N}_2\text{O}^{\text{bulk}}$ (¹⁵N abundance in N₂O), dual isotopes ($\delta^{15}\text{N}$, $\delta^{18}\text{O}$) and site preference (SP) are the most effective methods currently. $\delta^{15}\text{N}_2\text{O}^{\text{bulk}}$ is useful in tracing substrates yielding N₂O, but it is helpless to make accurate differentiation between nitrification and denitrification; The dual isotopes, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$, introduce O atom sources in partitioning N₂O source and this method could roughly apportion N₂O from nitrification and denitrification. However, expensive equipment requirements and the inherent uncertainties on $\delta^{18}\text{O}$ determination owing to few data available hamper its accuracy and applicability on distinguishing nitrification and denitrification. Site preference (SP) provides new insight on distinguishing contribution of nitrification and denitrification on N₂O emission and results in more accuracy results. We summarized that the average values of $\delta^{15}\text{N}_2\text{O}^{\text{bulk}}$, $\delta^{18}\text{O}$ and SP during denitrification, which were -29.29% , 19.78% , and 2.43% , respectively, are much lower than the corresponding values during nitrification, which were -7.87% , 48.03% and 24.14% , respectively.

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1. Introduction

Nitrous oxide (N₂O), a relatively stable greenhouse gas, has stronger warming potential than CO₂ and CH₄ [1], and its long-term presence in atmosphere would exhaustively deplete O₃ by photochemical reaction and draw secondary harms to biosphere on earth [2]. The preindustrial atmospheric N₂O content was 270 ng/L, but it has been increasing since 1750 at the rate of 0.2%–0.3%, and reached to 324 ng/L in 2011 [3,4]. If nothing is done to curb N₂O emission, atmospheric N₂O content is estimated to 350–400 ng/L in 2050, exceeding the current concentration by 83%, and N₂O would exert as the overwhelming O₃ consumer [5]. Soils

are the largest contributors to N₂O emissions, with 6.0 Tg/a from natural soils and 4.2 Tg/a from agricultural soils [6]. Despite extensive studies on N₂O produced by nitrification and denitrification, apportionment of N₂O to these two routes is ambiguous.

Microorganism-induced nitrification and denitrification are the main processes yielding N₂O, representing 90% of the total N₂O in global atmosphere [7]. N₂O is the precursor when N₂ produce is ascribed to nitrate denitrification, and is the intermediate during hydroxylamine oxidation [8,9] (Fig. 1). Generally, aerobic condition facilitates nitrification and ammonia in this situation is easily converted into nitrate by oxidation, which include two stages. The first stage, ammonia is oxidized to nitrite and N₂O simultaneously by nitrite bacteria. The second stage, nitrite is shifted into nitrate by nitrobacteria. Denitrification is to reduce nitrate to N₂ and is regarded as the main soil N₂O source [10].

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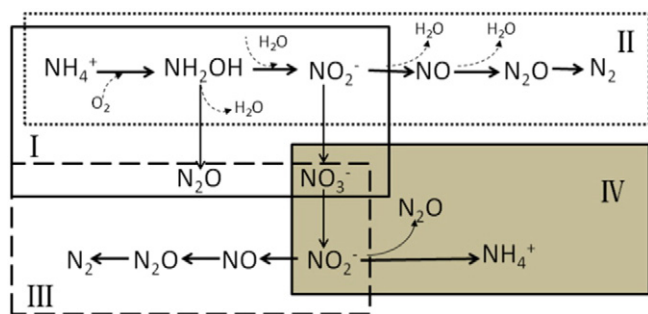


Fig. 1. N_2O production in soils. I is the nitrification process, II is the nitrifier denitrification process; III is the typical denitrification process, and IV is the nitrate ammonification process. In I and II, O_2 or H_2O in the arc represent O atom input or output during nitrification and nitrifier denitrification processes.

Denitrification generally occurs under anaerobic conditions, and three models are involved: 1) is defined as denitrifier denitrification, that is to say that denitrifying bacteria successively reduce NO_3^- , NO_2^- , NO and N_2O to N_2 , and N_2O is the by-product and might emit out from soil; 2) is the aerobic denitrification by heterotrophic nitrifiers, namely that some heterotrophic nitrifiers like *Pseudomonas denitrificans* can induce both nitrification and denitrification under aerobic conditions, and N_2O is produced as intermediate or precursor; 3) is nitrifier-denitrification, means that ammonia or hydroxylamine is firstly oxidized into NO_2^- , with complicating reduce into NO , N_2O and N_2 in a gradient. Such nitrifiers are *Nitroso monaseuropaea*, *Nitro sospiramultiformis*, and *methylosinus trichosporium* [11]. Recently, some marine ammonia oxidizing archaea also could emulate the nitrifier-denitrification process [12].

In this respect to N_2O yield in soil, many complex mechanisms are involved, and both nitrification or denitrification contribute to N_2O emission, which is concomitantly regulated by soil moisture [16,29], oxidation reduction potential [12,13], microbial communities and activities [14–16], organic carbon amounts and qualities, nutrients (N, P, S) availability [17] and so on. Elusive shifting between nitrification and denitrification arise confusions on differentiating N_2O source in soil. Increasing atmospheric nitrogen deposition from fuel consumption and agriculture fertilization, in conjunction with climate change, has greatly changed global nitrogen biogeochemistry, motivated or impeded N_2O emission. So, it is obligate to establish the relative contribution of nitrification and denitrification to N_2O yield.

The conventional and traditional technologies are helpless in distinguishing contributions of nitrification from denitrification to soil N_2O fluxes. Some incubation employed inhibitor (acetylene) to eliminate influences of nitrification as much as possible [18,19]. However, there are distinct interspaces. On one hand, there is no pure aerobic or anaerobic conditions allowing alternative nitrification or denitrification in natural conditions, and on the other hand, nitrification and denitrification often occur simultaneously and inter-shifting is ubiquitous. Soil environment in natural conditions are generally obviously derived from incubation experiments, drawing incomparable and questionable conclusions. Thereby, how to partition nitrification and denitrification contributions on N_2O yield under natural conditions is the pre-requisite to decipher nitrogen biogeochemistry, and stable isotopic technologies have provided promising approaches to enhance our knowledge on this item.

1.1. $\delta^{15}\text{N}_2\text{O}^{\text{bulk}}$ fingerprint N_2O from different substances

Despite extensive studies on N_2O emission labeled by ^{15}N , it still proved difficult to quantify contribution of nitrification and denitrification on soil N_2O . One issue raised in previous work was that controlled soil conditions must be involved by regulating soil moisture or adding inhibitor when only ^{15}N abundance in N_2O bulk ($\delta^{15}\text{N}_2\text{O}^{\text{bulk}}$) was

used. Li et al. [20] reported that, by signature forest and grassland soil using ^{15}N , both nitrification and denitrification contributed to soil N_2O emission under oxygen-abundant conditions, of which nitrification process was prevail and yielded about 53.1%–72.0% N_2O . $\delta^{15}\text{N}$ in different substrate, such as chemical fertilizer, natural soil, arable soil and manure (Fig. 2), often differ greatly. During N_2O formation, bond energy depends on $^{15}\text{N-M}$ or $^{14}\text{N-M}$ (M is the iron connecting N atom) and thereby would induce variation of $\delta^{15}\text{N}_2\text{O}^{\text{bulk}}$ from different soil texture or matrix (Table 1), and $\delta^{15}\text{N}_2\text{O}^{\text{bulk}}$ is an effective indicator to fingerprint soil matrix. Nitrification and denitrification had different impacts on $\delta^{15}\text{N}_2\text{O}^{\text{bulk}}$. When NO_x is reduced, $^{14}\text{NO}_x$ would be preferentially reduced because of the $^{14}\text{N-O}$ bond is easily broken by less energy, inducing exhausted ^{15}N in N_2O produced [21]. However, apportion nitrification and denitrification on N_2O flux could not be carried out just depending on $\delta^{15}\text{N}_2\text{O}^{\text{bulk}}$ under natural conditions up to now.

1.2. Dual isotopes fingerprint of N_2O

One of the most obvious drawbacks using $\delta^{15}\text{N}^{\text{bulk}}$ to differentiate denitrification and nitrification contributions on N_2O emission is that it is inefficient to trace N_2O yield during nitrifier-denitrification processes. So, another important atom, oxygen, is taken into account to further fingerprint N_2O source, and it seemed as be a promising approach.

N_2O is the most important intermediate during nitrification and denitrification. The dual isotopes of N_2O molecular, ^{15}N and ^{18}O , could impress N and O sources and abundance variation during N_2O formation, and then is able to distinguish nitrification from denitrification. O atom in N_2O molecular come from H_2O and soil air with different $\delta^{18}\text{O}$ abundance, which are +23.5‰ in soil air and –10‰ in soil H_2O [24], and this provides the theoretical basis for dual isotopes fingerprint technology. During nitrification processes, O atoms come from both soil air and soil H_2O [23], and $\delta^{18}\text{O}$ abundance in N_2O from nitrification occurring in aerobic environment ranked from +13‰ to +35‰ compared with VSMOW (Vienna Standard Mean Ocean Water), respectively [25]. This is also proved in N_2O from marine. Ammonia oxidation by ammonia oxidizing archaea (AOA) is the most important pathway for N_2O formation in marine, and $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ in N_2O from this process are much higher than those from N_2O produced by ammonia oxidizing bacteria (AOB). However, in spite of the different biological mechanisms for N_2O , O atoms in N_2O formed in the two processes mentioned above are resemble, of which water and air supply 50% of total O atoms, respectively [12,26] (Fig. 2). For denitrification, $\delta^{18}\text{O}$ in N_2O formed are generally much lower than that in soil NO_3^- , indicating that most O in N_2O come from soil H_2O during NO_3^- reduction into N_2O [23]. However, there is still some elusive inconformity between different researches. Frame et al. [27] reported that O atom of N_2O from NH_2OH oxidation just originates from soil air, and O atom of N_2O from nitrifier-

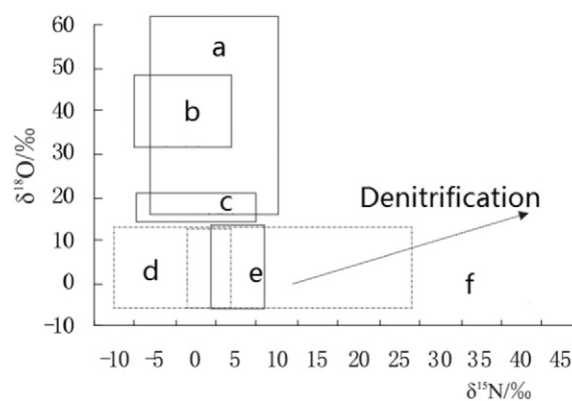


Fig. 2. $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ in different substrates. a, nitrate in rainfall; b, nitrate atmospheric deposition in virgin soil, c, nitrate fertilizer; d, NH_4 in fertilizer and rain; e, Nitrogen in soil, f, animal waste.

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