Review

Nitrous Oxide Emissions from Paddies:
Understanding the Role of Rice Plants

Arbindra Timilsina 1,2,*, Fiston Bizimana 1,2, Bikram Pandey 2,3, Ram Kailash Prasad Yadav 4, Wenxu Dong 1 and Chunsheng Hu 1,*

1 Key Laboratory of Agricultural Water Resources, Hebei Key Laboratory of Soil Ecology, Center for Agricultural Resources Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Shijiazhuang 050021, China; bizifis@gmail.com (F.B.); dongwx@sjziam.ac.cn (W.D.)
2 University of Chinese Academy of Sciences, Beijing 100049, China; bikram@cib.ac.cn
3 Key Laboratory of Mountain Ecological Restoration and Bio-resource Utilization and Ecological Restoration Biodiversity Conservation Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, Sichuan, China
4 Central Department of Botany, Tribhuvan University, Kirtipur 44613, Kathmandu, Nepal; rkp.yadav@cdbtu.edu.np
* Correspondence: arbintms@sjziam.ac.cn (A.T.); cshu@sjziam.ac.cn (C.H.); Tel.: +8631185814360 (C.H.)

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Abstract: Paddies are a potential source of anthropogenic nitrous oxide (N₂O) emission. In paddies, both the soil and the rice plants emit N₂O into the atmosphere. The rice plant in the paddy is considered to act as a channel between the soil and the atmosphere for N₂O emission. However, recent studies suggest that plants can also produce N₂O, while the mechanism of N₂O formation in plants is unknown. Consequently, the rice plant is only regarded as a channel for N₂O produced by soil microorganisms. The emission of N₂O by aseptically grown plants and the distinct dual isotopologue fingerprint of plant-emitted N₂O, as reported by various studies, support the production of N₂O in plants. Herein, we propose a potential pathway of N₂O formation in the rice plant. In rice plants, N₂O might be formed in the mitochondria via the nitrate–nitrite–nitric oxide (NO₃–NO₂–NO) pathway when the cells experience hypoxic or anoxic stress. The pathway is catalyzed by various enzymes, which have been described. So, N₂O emitted from paddies might have two origins, namely soil microorganisms and rice plants. So, regarding rice plants only as a medium to transport the microorganism-produced N₂O might be misleading in understanding the role of rice plants in the paddy. As rice cultivation is a major agricultural activity worldwide, not understanding the pathway of N₂O formation in rice plants would create more uncertainties in the N₂O budget.

Keywords: anoxia; hypoxia; mitochondria; nitric oxide; nitrous oxide; paddy; potential pathway; rice plant

1. Introduction

Nitrous oxide (N₂O) is a major anthropogenic greenhouse gas and the single most important contributor to stratospheric ozone depletion [1,2]. It accounts for approximately 6% of the enhanced global warming effect [3]. Among the anthropogenic sources of N₂O, the agriculture sector represents the largest source [4]. Rice (Oryza sativa) farming plays an important role in the agricultural sector as it is a staple food for one-half of the world’s population [5]. Additionally, rice farming occupies about 158.5 million hectares of the world’s arable land and is one of the most important economic activities that provides a livelihood to millions of people [5]. Paddies are also a contributor of N₂O to the atmosphere [5,6]. Moreover, the paddy utilizes one-seventh of the
nitrogen (N) fertilizer and one-third of irrigation globally [7], making a more potent zone of N$_2$O formation, as the N fertilizer application [8] and irrigation management practices [9] contribute significantly to N$_2$O emissions. So, global N$_2$O emissions from the paddy might increase considerably [10]. Thus, it is necessary to understand the mechanisms of N$_2$O production in the paddy for necessary steps to be taken towards mitigation strategies for the global warming effect.

N$_2$O in the paddy is emitted by the soil [10,11] and the rice plant [12,13]. The processes involved in the soil are well studied [10,11]. However, processes involving the plant are overlooked and the N$_2$O emitted by the rice plants in paddies is considered to be produced by soil microorganisms, with the rice plant hypothesized to act as a channel to emit it into the atmosphere [12,13]. However, studies hypothesizing rice plants as a channel to emit N$_2$O [12,13] have only measured the flux from the soil and plants and concluded that rice plants are not a source of N$_2$O. Recently, Lenhart et al., (2019) [14] reported the distinct dual isotopocule fingerprint of N$_2$O ($\delta^{15}$N, $\delta^{18}$O) emitted from the plant Miscanthus sinensis, and suggested that plants are a natural source of N$_2$O. So, in our opinion, measuring only the N$_2$O fluxes from the soil and the rice plant may not be sufficient to prove that rice plants are not a source of N$_2$O. The results of Smart and Bloom (2000) [15] do not support the hypothesis that N$_2$O emitted by wheat plants is produced by microorganisms and via the transpiration process. To elucidate this, we suggest the use of the $^{15}$N natural abundance method by injecting $^{15}$N-labelled N$_2$O into the soil zone and, subsequently, measuring the fluxes and the $^{15}$N natural abundance of N$_2$O from the soil and plants to reveal whether the N$_2$O emissions are emitted either from soil and plants transport them, or whether N$_2$O emissions can also be produced from plant cells. Furthermore, the rice plants should be aseptically grown in a controlled hydroponic solution with a regulated O$_2$ partial pressure, and NO$_3$ and NH$_4$ concentrations and, subsequently, the N$_2$O fluxes should be measured. Moreover, recent studies have shown that various species of plants can produce N$_2$O and emit it into the atmosphere [14–16], however, the mechanism of N$_2$O formation is not clearly understood. Therefore, considering rice plants only as a channel for soil-produced N$_2$O might mislead understanding of the role of rice plants in N$_2$O emission, as it is a conclusion based on studies that just measured the N$_2$O fluxes from rice soil and the rice plant.

There are reports of both higher [17,18] and lower flux rates of N$_2$O from paddies [19,20]. The high or low emissions of N$_2$O from paddies depend on the management practices [18–20]. The N fertilizer application rate and water level are major factors controlling the N$_2$O emissions from paddies [8,20]. For example, a meta-analysis by Zou et al. (2007) [19] revealed that under a continuous flooding system, N$_2$O is emitted only after drainage, whereas flooding-midseason drainage-reflooding management triggers a substantial N$_2$O emission. Moreover, flooding-midseason drainage-reflooding moist intermittent irrigation practices without waterlogging trigger a threefold higher N$_2$O emission than the flooding-midseason drainage-reflooding management practice. A recent meta-analysis [9] reported a 105% increase in N$_2$O emission with non-continuous flooding management rather than continuous flooding. Similarly, midseason drainage and N application significantly increased the N$_2$O emissions from the paddies [8]. From these observations of the meta-analyses, it can be concluded that water level management and N application significantly affect the N$_2$O fluxes from the paddies. Interestingly, water level management [13] and nitrogen fertilizer application [12] also significantly increased the N$_2$O emission from the rice plants and, in both cases, the emissions of N$_2$O were higher from rice plants than from the soil–water surface [12,13]. These results highlight the role of rice plants in N$_2$O emissions from paddies.

To mitigate the effects of global warming and ozone depletion effectively, a good understanding of all of the sources of N$_2$O and the regulating factors is crucial. There have been numerous studies on paddies regarding their N$_2$O emissions [8,12,13,20,21], however, no study has highlighted the role of the rice plant as a source of N$_2$O. Thus, it is essential to explore the role of the rice plant in paddies—that is, whether it acts as a source of or a medium to channel N$_2$O.
production of \( \text{N}_2\text{O} \) in ascetically grown plants [16,22] suggests \( \text{N}_2\text{O} \) can be produced by even eukaryotic plant cells. Moreover, based on the distinct dual isotopocule fingerprint of plant-emitted \( \text{N}_2\text{O} \), Lenhart et al. (2019) [14] proposed that plants are a natural source of \( \text{N}_2\text{O} \). So, the rice plant might also be a source of \( \text{N}_2\text{O} \). Now arises the question of what the mechanism of \( \text{N}_2\text{O} \) formation in the rice plant cell is, if the rice plant is a source of \( \text{N}_2\text{O} \). Due to the unknown mechanisms of \( \text{N}_2\text{O} \) formation in the plant cell, the rice plant might be regarded as just a medium to transport the soil microorganism-produced \( \text{N}_2\text{O} \). Therefore, to elucidate the origin of \( \text{N}_2\text{O} \) emitted from paddies, we propose a potential pathway of \( \text{N}_2\text{O} \) formation within the rice plant.

2. Potential Pathway of \( \text{N}_2\text{O} \) Formation in Rice Plants

Studies on plants’ \( \text{N}_2\text{O} \) emissions, involving the usage of the \( ^{15}\text{N} \) isotope labeling method, have shown nitrate (\( \text{NO}_3 \)) as a precursor to \( \text{N}_2\text{O} \) formation, but not ammonium (\( \text{NH}_4 \)) [8,14–16,22–24]. Furthermore, aseptically grown plants and axenic algal cells, when supplied with \( ^{15}\text{N}-\text{NO}_2 \), have also been found to produce \( ^{15}\text{N}-\text{N}_2\text{O}_{\text{bulk}} \) [16,22,25]. In addition, eukaryotic cells, when supplied with \( ^{15}\text{N}-\text{NO} \), produce \( ^{15}\text{N}-\text{N}_2\text{O}_{\text{bulk}} \) via a reduced form of cytochrome c oxidase [26]. Therefore, \( \text{NO}_3, \text{NO}_2 \), and nitrous oxide (\( \text{NO}_2 \)) are the sources of \( \text{N}_2\text{O} \) in eukaryotic cells. Thus, \( \text{N}_2\text{O} \) formation in the cells of rice plants might occur via the \( \text{NO}_3\text{-NO}_2\text{-NO} \) pathway.

Nitric oxide (\( \text{NO} \)), being a signaling molecule, is formed at the cellular level in every eukaryotic organism [27]. There are, mainly, two potential pathways of \( \text{NO} \) formation in cells, namely the oxidative and reductive pathways. The oxidative pathway is L-arginine-dependent and occurs when the oxygen concentration in the cells is sufficient, whereas the reductive pathway is \( \text{NO}_3 \) and \( \text{NO}_2 \)-dependent and occurs at a low oxygen concentration in the cells [28,29]. Briefly, the reductive pathway can occur in the hypoxic or anoxic cell environment, via \( \text{NO}_3\text{-NO}_2\text{-NO} \), and is catalyzed by various enzymes [27,28,30]. For example, \( \text{NO}_3 \) taken by the plant root is reduced to \( \text{NO}_2 \) in the cytoplasm by the cytoplasmic nitrate reductase (NR) [29]. Hypoxia caused by flooding in the root may increase [31], decrease [32], or have no effect [33] on \( \text{NO}_3 \) uptake from the soil. However, flood-tolerant species such as rice might have higher NR activities than flood-sensitive species [34]. During hypoxia and anoxia, the \( \text{NO}_3 \) and \( \text{NH}_4 \) assimilation to amino acids strongly decreases [35]. So, \( \text{NO}_2 \) formed in the cytosol, due to the reduction in \( \text{NO}_3 \) by NR, is transported to the mitochondria by a protein similar to that in the chloroplast [30,36]. Moreover, the mitochondrial inner membrane anion channel may also be responsible for the transport of nitrite to the mitochondria [30].

It is evident that the mitochondrion is a site of \( \text{NO}_3 \) reduction [30,37]. \( \text{NO}_3 \) in the mitochondria is reduced to \( \text{NO} \), and the process is pronounced under hypoxic to anoxic stress [30,38,39] and is more favorable at a lower pH [40,41]. Accordingly, Klepper (1987) [42] and Rockel et al. (2002) [43] reported a high emission of \( \text{NO} \) from plant leaves after the supply of \( \text{NO}_3 \) under anaerobic conditions. The reduction process in the mitochondria is catalyzed by various electron transport chains (ECTs) [39,44]. For example, complex III [45] (Benamar et al., 2008), cytochrome c [44,46], and alternative oxidase (AOX) [47] can catalyze the reduction of \( \text{NO}_3 \) to \( \text{NO} \) in the mitochondria. The \( \text{NO} \) subsequently formed is further reduced to \( \text{N}_2\text{O} \) by a reduced form of eukaryotic cytochrome c oxidase [26,48]. The conversion of \( \text{NO} \) into \( \text{N}_2\text{O} \) by a reduced form of cytochrome c oxidase is more favorable at low levels of both \( \text{NO} \) and \( \text{O}_2 \) [48]. Cytochrome c oxidase in aerobic organisms is considered to be evolved from bacterial denitrifying enzymes [49]. Therefore, it is believed that, under the condition of less oxygen in cells, the enzyme exhibits some rudimentary nitrite and nitric oxide reductase activities [26,48,50]. Moreover, quinone in the mitochondria can also catalyze the reduction of \( \text{NO} \) to \( \text{N}_2\text{O} \) [51–53].
Figure 1. The potential pathway of N\textsubscript{2}O formation in rice plants under hypoxic conditions. ?? sign represents the involvement of two or more enzymes [9,23,27,44,48,52].

Therefore, the processes, like the reduction of NO\textsubscript{2} to NO and NO to N\textsubscript{2}O, are favorable under hypoxic and anoxic conditions in the mitochondria of plants. As discussed in the introduction section, the emission of N\textsubscript{2}O from paddies is very high under non-continuous flooding, midseason drainage, and reduced irrigation with midseason drainage management practices. All of these practices alter the oxygen (O\textsubscript{2}) level significantly in the root cells of the rice plant [54,55], which may create a favorable zone for N\textsubscript{2}O formation. Laboratory-based measurements also showed approximately 82–92% of N\textsubscript{2}O emissions from rice plants under soil flooded conditions, whereas 7–24% were found under unsaturated soil water conditions [13]. Along with O\textsubscript{2}, NO\textsubscript{3} also plays an important role in N\textsubscript{2}O emission from plants, as NO\textsubscript{3} is the precursor to N\textsubscript{2}O production in plants [14–16]. The role of O\textsubscript{2} in the proposed pathway is supported by high NO\textsubscript{2} and NO emissions from the soybean plant [56] and N\textsubscript{2}O [13] from the rice plant under anaerobic conditions. Furthermore, the role of NO\textsubscript{3} in the proposed pathway is supported by the emission of \textsuperscript{15}N-labelled NO and \textsuperscript{15}N-N\textsubscript{2}O\textsubscript{bulk} from plants when supplied with \textsuperscript{15}N-NO\textsubscript{3} [23]. Therefore, NO\textsubscript{3} concentration in the root zone and cells might affect the N\textsubscript{2}O emission from rice plants, as NO\textsubscript{3} uptake in the plant increases when its concentration in the soil solution is increased [57,58]. It might be the reason that soil fertilization with KNO\textsubscript{3} greatly enhances N\textsubscript{2}O emission from rice plants [12]. Thus, N\textsubscript{2}O emitted by rice plants might be formed in hypoxic or anoxic mitochondria, as shown in Figure 1. It further suggests the existence of an incomplete denitrification (NO\textsubscript{3}-NO\textsubscript{2}-NO-N\textsubscript{2}O) pathway in the plant cells [16] when the oxygen level in the cells declines. This is further supported by the production of N\textsubscript{2}O in plants after the supply of NO\textsubscript{3} and NO\textsubscript{2}, but not NH\textsubscript{4} [14–16,22–24]. As there are many reports that the rice plant emits a substantial amount of N\textsubscript{2}O [12,13], and that within the plant cell there exists evidence of the NO\textsubscript{3}-NO\textsubscript{2}-NO-N\textsubscript{2}O pathway [26,29,30,37,48], this suggests that rice plants are a source of N\textsubscript{2}O. So, considering rice plants only as a channel for soil-produced N\textsubscript{2}O might create a gap in understanding N\textsubscript{2}O emission from paddies.

3. Why It Is a Challenge to Understand the Role of Rice Plants with Current Approaches to Methods from Field Studies?

Current methodologies involved in field-based measurements of N\textsubscript{2}O emissions from paddies include closed static chamber-based methods [18,59] and the micrometerological approach (the eddy covariance method) [10,60,61]. These methods include both soil and rice plants in the same system [10,18,59–61], making it difficult to evaluate the role of rice plants. However, for other plants with bigger stems (like trees), recent studies have developed separate chambers that help to capture the fluxes of N\textsubscript{2}O by avoiding soil N\textsubscript{2}O emissions [62]. The chamber used for trees cannot be used for crop species, like rice. The studies taken in field conditions [18,59–61] have not developed such chambers that could separately quantify the N\textsubscript{2}O emitted from the rice plant and the rice soil. In this regard, static chambers that could be used for crops like the rice plant should be developed and the N\textsubscript{2}O fluxes from the rice plant and the rice soil (microbial) should be measured separately. This limitation has made it difficult to evaluate the contribution of the rice plants to the total contribution from the paddy and the N\textsubscript{2}O budget from rice plants remains unsolved. Although it
might be a challenge to prove that all N\textsubscript{2}O emitted by the plants in field conditions is from either soil microorganisms, plants, or both, injecting \(\delta^{15}\text{N}-\text{N}_2\text{O}_{\text{bulk}}\) into the soil profile and subsequently measuring the N\textsubscript{2}O fluxes in the soil and the rice plants might help to distinguish the source. Additionally, to understand the role of the rice plants in the paddy, we suggest measuring the isotopocule fingerprint of N\textsubscript{2}O (\(\delta^{15}\text{N}, \delta^{15}\text{N}_{\text{sp}}, \text{and} \delta^{18}\text{O}\)) emitted from rice plants and rice soil, as isotope analysis methods are a more powerful tool than the flux measurement methods to distinguish the source of the N\textsubscript{2}O [63].

At the plant’s cellular level, the NO\textsubscript{3}-NO\textsubscript{2}-NO-N\textsubscript{2}O pathway, as represented in Figure 1 operates during limited oxygen in cells and in the presence of NO\textsubscript{3}, NO\textsubscript{2} [26,29,30,37,48]. Similarly, in the rice soil the microbial pathway of denitrification (NO\textsubscript{3}-NO\textsubscript{2}-NO-N\textsubscript{2}O-N\textsubscript{2}) occurred during limited oxygen conditions and in the presence of NO\textsubscript{3} and NO\textsubscript{2} [10,64]. So, both in the rice plant and the rice soil, the pathway of N\textsubscript{2}O formation occurred during the limited oxygen conditions, making the process appear only to occur in the known site, i.e., the soil. As the N\textsubscript{2}O flux measurement was done with the help of static chambers that captured the N\textsubscript{2}O fluxes in the soil and the rice plant at bulk [18,59], this masked the role of the rice plant. However, as described in Section 2, the NO\textsubscript{3}-NO\textsubscript{2}-NO-N\textsubscript{2}O pathway existed in the plant cells during hypoxia and anoxia and this pathway in the rice plant cells was overlooked.

4. Role of NO\textsubscript{3}, NO\textsubscript{2} and NO during Hypoxia and Anoxia Tolerance

Rice farming needs frequent irrigation, resulting in hypoxic and anoxic conditions in the root zone. Under such conditions, oxygen concentration in the cells is too low to support aerobic respiration and may cause energy deficit and, ultimately, cell death [65]. Various studies have shown that NO\textsubscript{3} fertilization improves tolerance to low oxygen conditions in plants [31,66]. During hypoxia, and in the absence of NO\textsubscript{3}, the plants’ growth is significantly disturbed [31]. So, for crop species like the rice plant, which is frequently subjected to hypoxia and anoxia, NO\textsubscript{3} might play important role in improving tolerance to low oxygen.

Oxygen deprivation affects the plant mitochondria [67,68]. Nitrate and nitrite are shown to have a protective effect on mitochondria under oxygen deprivation [68]. Hypoxia strongly decreases the NO\textsubscript{2} reduction to NH\textsubscript{4} and the NH\textsubscript{4} assimilation to amino acids [35]. As NO\textsubscript{2} assimilated to NH\textsubscript{4} is reduced during hypoxia [35], it is accumulated in the cytosol [30,65] or released to the external medium [66]. The accumulated NO\textsubscript{2} can serve as an alternative electron acceptor at mitochondrial electron transport chain (ETC), with NO as a significant product of the reaction [52]. Moreover, the pathway of NO formation through NO\textsubscript{3}-NO\textsubscript{2} in the mitochondria contributes to the ATP synthesis [33,65,69]. The NO\textsubscript{3}-NO\textsubscript{2}-derived ATP production in mitochondria can make an important contribution to hypoxia survival [70]. Although NO is a signaling molecule [27–30], an excess of NO in the mitochondria can be toxic to the cell and may result in the death of the cell [71]. Although N\textsubscript{2}O is a potent greenhouse gas, the conversion of NO to N\textsubscript{2}O by cytochrome c oxidase [26,48] might play an important role in maintaining the integrity of mitochondria during limited oxygen conditions, as an excess of NO can be toxic to the cell [71]. So, the high emissions of N\textsubscript{2}O from rice plants during limited oxygen conditions, as reported by Yan et al., (2000) [13] might be an efficient mechanism to reduce the toxicity of NO and to maintain the integrity of the mitochondria under hypoxia and anoxia, therefore, this should be a matter of further research.

5. Conclusions and Future Perspectives

In conclusion, NO\textsubscript{3} is reduced to NO\textsubscript{2} in the cytosol, and NO\textsubscript{2} reduction to NO, along with NO reduction to N\textsubscript{2}O, occurs in the mitochondria of rice plants. The pathway of N\textsubscript{2}O formation is formed under hypoxic or anoxic conditions, and the water management practice may often cause hypoxia and anoxia in the rice root, making the rice plant a potent source of N\textsubscript{2}O. Therefore, we suggest that, while studying N\textsubscript{2}O emission from paddies, the potential pathway of N\textsubscript{2}O formation in rice plants should be explored. To date, the rice plant is hypothesized to be a medium to transport the N\textsubscript{2}O produced in the soil by microorganisms. However, recent studies suggest that plants can produce N\textsubscript{2}O, which may be during the metabolism of NO to N\textsubscript{2}O in plant mitochondria.
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[30], as shown in this study. The significant amount of N₂O emitted from rice plants suggests the possibility of the existence of the proposed pathway of N₂O formation in rice plants. Furthermore, the NO₃-NO₂-derived NO formation pathway in the hypoxic and anoxic rice plant’s mitochondria helps the ATP synthesis and develop a tolerance to limited oxygen conditions. The rice plant, being an anoxia-tolerant plant, in which NO₂ dependent NO production in mitochondria was sustained for almost twice as long as barley [67], suggests that these plants might be a more potent producer of N₂O. However, to date, N₂O emitted from the paddy is regarded as being produced by various microbes [10,21] and the N₂O formation pathway in plants is neglected. Future studies are certainly required to elaborate on the proposed pathway of N₂O formation in the rice plant. Furthermore, the transport of soil-produced N₂O by plants, as hypothesized by many experiments [12,13], is not supported because there are no emissions of N₂O by plants when supplied with NH₄ and during a high rate of N₂O production in the rhizosphere [15]. So, it would be misleading to understand the N₂O emitted by the rice plant to be soil microorganism-produced. Understanding the role of the rice plant is necessary for further strategies to be taken to mitigate N₂O emissions from the paddy. Quantifying the dual isotopocule fingerprint of N₂O, along with the molecular-based studies, would provide clear insights into the proposed mechanism. Therefore, the extraction of rice mitochondria and the measuring of NO and N₂O emissions under different conditions of O₂ and NO₃⁻ would provide insights into the N₂O pathway. Besides, the reduced form of cytochrome c oxidase and quinone are found to catalyze NO reduction to N₂O and, therefore, their roles in rice plants should be investigated in detail. Moreover, to differentiate the soil sources from the plant source and quantify the portion of soil and plant contributions to total emissions will be a big challenge with the current approach to the methodology used. The development of static chambers that could capture the fluxes of N₂O from the rice plant and separate the soil emissions would help to evaluate the rice plant’s role in the total emissions.

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