


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: arbinms@sjziam.ac.cn (A.T.); cshu@sjziam.ac.cn (C.H.); Tel.: +8631185814360 (C.H.)

Received: 20 December 2019; Accepted: 29 January 2020; Published: 2 February 2020



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 isotopocule 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 N₂O formation, as the N fertilizer application [8] and irrigation management practices [9] contribute significantly to N₂O emissions. So, global N₂O emissions from the paddy might increase considerably [10]. Thus, it is necessary to understand the mechanisms of N₂O production in the paddy for necessary steps to be taken towards mitigation strategies for the global warming effect.

N₂O 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 N₂O 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 N₂O [12,13] have only measured the flux from the soil and plants and concluded that rice plants are not a source of N₂O. Recently, Lenhart et al., (2019) [14] reported the distinct dual isotopic fingerprint of N₂O ($\delta^{15}\text{N}$, $\delta^{15}\text{N}^{\text{sp}}$ and $\delta^{18}\text{O}$) emitted from the plant *Miscanthus sinensis*, and suggested that plants are a natural source of N₂O. So, in our opinion, measuring only the N₂O fluxes from the soil and the rice plant may not be sufficient to prove that rice plants are not a source of N₂O. The results of Smart and Bloom (2000) [15] do not support the hypothesis that N₂O emitted by wheat plants is produced by microorganisms and via the transpiration process. To elucidate this, we suggest the use of the ¹⁵N natural abundance method by injecting ¹⁵N-labelled N₂O into the soil zone and, subsequently, measuring the fluxes and the ¹⁵N natural abundance of N₂O from the soil and plants to reveal whether the N₂O emissions are emitted either from soil and plants transport them, or whether N₂O emissions can also be produced from plant cells. Furthermore, the rice plants should be aseptically grown in a controlled hydroponic solution with a regulated O₂ partial pressure, and NO₃ and NH₄ concentrations and, subsequently, the N₂O fluxes should be measured. Moreover, recent studies have shown that various species of plants can produce N₂O and emit it into the atmosphere [14–16], however, the mechanism of N₂O formation is not clearly understood. Therefore, considering rice plants only as a channel for soil-produced N₂O might mislead understanding of the role of rice plants in N₂O emission, as it is a conclusion based on studies that just measured the N₂O fluxes from rice soil and the rice plant.

There are reports of both higher [17,18] and lower flux rates of N₂O from paddies [19,20]. The high or low emissions of N₂O from paddies depend on the management practices [18–20]. The N fertilizer application rate and water level are major factors controlling the N₂O emissions from paddies [8,20]. For example, a meta-analysis by Zou et al. (2007) [19] revealed that under a continuous flooding system, N₂O is emitted only after drainage, whereas flooding-midseason drainage-reflooding management triggers a substantial N₂O emission. Moreover, flooding-midseason drainage-reflooding moist intermittent irrigation practices without waterlogging trigger a threefold higher N₂O emission than the flooding-midseason drainage-reflooding management practice. A recent meta-analysis [9] reported a 105% increase in N₂O emission with non-continuous flooding management rather than continuous flooding. Similarly, midseason drainage and N application significantly increased the N₂O emissions from the paddy [11]. Another meta-analysis, based on the comparison between flooding irrigation and non-flooding irrigation (reduced irrigation with midseason drainage) and N fertilizer input, showed an increase in N₂O emission of 84.4% and 167.3%, respectively [8]. From these observations of the meta-analyses, it can be concluded that water level management and N application significantly affect the N₂O fluxes from the paddies. Interestingly, water level management [13] and nitrogen fertilizer application [12] also significantly increased the N₂O emission from the rice plants and, in both cases, the emissions of N₂O were higher from rice plants than from the soil–water surface [12,13]. These results highlight the role of rice plants in N₂O emissions from paddies.

To mitigate the effects of global warming and ozone depletion effectively, a good understanding of all of the sources of N₂O and the regulating factors is crucial. There have been numerous studies on paddies regarding their N₂O emissions [8,12,13,20,21], however, no study has highlighted the role of the rice plant as a source of N₂O. 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 N₂O. The production of N₂O in aseptically

grown plants [16,22] suggests N_2O can be produced by even eukaryotic plant cells. Moreover, based on the distinct dual isotopocule fingerprint of plant-emitted N_2O , Lenhart et al. (2019) [14] proposed that plants are a natural source of N_2O . So, the rice plant might also be a source of N_2O . Now arises the question of what the mechanism of N_2O formation in the rice plant cell is, if the rice plant is a source of N_2O . Due to the unknown mechanisms of N_2O formation in the plant cell, the rice plant might be regarded as just a medium to transport the soil microorganism-produced N_2O . Therefore, to elucidate the origin of N_2O emitted from paddies, we propose a potential pathway of N_2O formation within the rice plant.

2. Potential Pathway of N_2O Formation in Rice Plants

Studies on plants' N_2O emissions, involving the usage of the ^{15}N isotope labeling method, have shown nitrate (NO_3) as a precursor to N_2O formation, but not ammonium (NH_4) [8,14–16,22–24]. Furthermore, aseptically grown plants and axenic algal cells, when supplied with $^{15}N-NO_2$, have also been found to produce $\delta^{15}N-N_2O^{bulk}$ [16,22,25]. In addition, eukaryotic cells, when supplied with $^{15}N-NO$, produce $\delta^{15}N-N_2O^{bulk}$ via a reduced form of cytochrome c oxidase [26]. Therefore, NO_3 , NO_2 , and nitrous oxide (NO) are the sources of N_2O in eukaryotic cells. Thus, N_2O formation in the cells of rice plants might occur via the NO_3-NO_2-NO pathway.

Nitric oxide (NO), being a signaling molecule, is formed at the cellular level in every eukaryotic organism [27]. There are, mainly, two potential pathways of 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 NO_3 and 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 NO_3-NO_2 , and is catalyzed by various enzymes [27,28,30]. For example, NO_3 taken by the plant root is reduced to 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 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 NO_2 and NH_4 assimilation to amino acids strongly decreases [35]. So, NO_2 formed in the cytosol, due to the reduction in 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 NO_2 reduction [30,37]. NO_2 in the mitochondria is reduced to 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 NO from plant leaves after the supply of 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 NO_2 to NO in the mitochondria. The NO subsequently formed is further reduced to N_2O by a reduced form of eukaryotic cytochrome c oxidase [26,48]. The conversion of NO into N_2O by a reduced form of cytochrome c oxidase is more favorable at low levels of both NO and 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 NO to N_2O [51–53].

Therefore, the processes, like the reduction of NO_2 to NO and NO to N_2O , are favorable under hypoxic and anoxic conditions in the mitochondria of plants. As discussed in the introduction section, the emission of N_2O 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_2) level significantly in the root cells of the rice plant [54,55], which may create a favorable zone for N_2O formation. Laboratory-based measurements also showed approximately 82–92% of

N_2O emissions from rice plants under soil flooded conditions, whereas 7–24% were found under unsaturated soil water conditions [13]. Along with O_2 , NO_3^- also plays an important role in N_2O emission from plants, as NO_3^- is the precursor to N_2O production in plants [14–16]. The role of O_2 in the proposed pathway is supported by high NO_2 and NO emissions from the soybean plant [56] and N_2O [13] from the rice plant under anaerobic conditions. Furthermore, the role of NO_3^- in the proposed pathway is supported by the emission of ^{15}N -labelled NO and $\delta^{15}N-N_2O^{bulk}$ from plants when supplied with $^{15}N-NO_3^-$ [23]. Therefore, NO_3^- concentration in the root zone and cells might affect the N_2O emission from rice plants, as NO_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_3 greatly enhances N_2O emission from rice plants [12]. Thus, N_2O 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_3^- - NO_2^- - NO - N_2O) pathway in the plant cells [16] when the oxygen level in the cells declines. This is further supported by the production of N_2O in plants after the supply of NO_3^- and NO_2^- , but not NH_4^+ [14–16,22–24]. As there are many reports that the rice plant emits a substantial amount of N_2O [12,13], and that within the plant cell there exists evidence of the NO_3^- - NO_2^- - NO - N_2O pathway [26,29,30,37,48], this suggests that rice plants are a source of N_2O . So, considering rice plants only as a channel for soil-produced N_2O might create a gap in understanding N_2O emission from paddies.

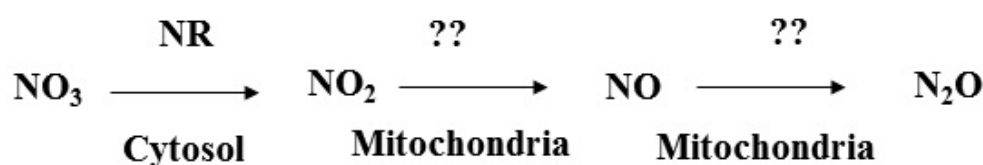


Figure 1. The potential pathway of N_2O formation in rice plants under hypoxic conditions. ?? sign represents the involvement of two or more enzymes [9,23,27,44,48,52].

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_2O emissions from paddies include closed static chamber-based methods [18,59] and the micrometeorological 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_2O by avoiding soil N_2O 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_2O 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_2O 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_2O budget from rice plants remains unsolved. Although it might be a challenge to prove that all N_2O emitted by the plants in field conditions is from either soil microorganisms, plants, or both, injecting $\delta^{15}N-N_2O^{bulk}$ into the soil profile and subsequently measuring the N_2O 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 isotopic fingerprint of N_2O ($\delta^{15}N$, $\delta^{15}N^{sp}$ and $\delta^{18}O$) 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_2O [63].

At the plant's cellular level, the NO_3^- - NO_2^- - NO - N_2O pathway, as represented in Figure 1 operates during limited oxygen in cells and in the presence of NO_3^- , NO_2^- [26,29,30,37,48]. Similarly, in the rice soil the microbial pathway of denitrification (NO_3^- - NO_2^- - NO - N_2O - N_2) occurred during limited oxygen conditions and in the presence of NO_3^- and NO_2^- [10,64]. So, both in the rice plant and the rice soil,

the pathway of N_2O 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_2O flux measurement was done with the help of static chambers that captured the N_2O 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_3 - NO_2 - NO - N_2O pathway existed in the plant cells during hypoxia and anoxia and this pathway in the rice plant cells was overlooked.

4. Role of NO_3 , NO_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_3 fertilization improves tolerance to low oxygen conditions in plants [31,66]. During hypoxia, and in the absence of NO_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_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_2 reduction to NH_4 and the NH_4 assimilation to amino acids [35]. As NO_2 assimilated to NH_4 is reduced during hypoxia [35], it is accumulated in the cytosol [30,65] or released to the external medium [66]. The accumulated NO_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_3 - NO_2 in the mitochondria contributes to the ATP synthesis [33,65,69]. The NO_3 - NO_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_2O is a potent greenhouse gas, the conversion of NO to N_2O 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_2O 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_3 is reduced to NO_2 in the cytosol, and NO_2 reduction to NO , along with NO reduction to N_2O , occurs in the mitochondria of rice plants. The pathway of N_2O 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_2O . Therefore, we suggest that, while studying N_2O emission from paddies, the potential pathway of N_2O formation in rice plants should be explored. To date, the rice plant is hypothesized to be a medium to transport the N_2O produced in the soil by microorganisms. However, recent studies suggest that plants can produce N_2O , which may be during the metabolism of NO to N_2O in plant mitochondria [30], as shown in this study. The significant amount of N_2O emitted from rice plants suggests the possibility of the existence of the proposed pathway of N_2O formation in rice plants. Furthermore, the NO_3 - NO_2 -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 NO_2 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 N_2O . However, to date, N_2O emitted from the paddy is regarded as being produced by various microbes [10,21] and the N_2O formation pathway in plants is neglected. Future studies are certainly required to elaborate on the proposed pathway of N_2O formation in the rice plant. Furthermore, the transport of soil-produced N_2O 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.

Author Contributions: A.T. and C.H. designed the concept. F.B., B.P., R.K.P.Y. and W.D. commented on the first draft of the manuscript. All the authors confirmed the final version of the manuscript. All authors have read and agreed to the published version of the manuscript

Funding: This study was supported by funding from the Key Program of National Natural Science Foundation of China (Grant No. 41530859) and National Key Research and Development Program of China (2016YFD0800102-4 and 2018YFC0213300-01).

Acknowledgments: Arbindra Timilsina is thankful to CAS-TWAS fellowship for granting the PhD scholarship. We are also grateful to Prof. Oene Oenema for his valuable comments. We are also thankful to the reviewers for their constructive comments and suggestions.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Ravishankara, A.R.; Daniel, J.S.; Portmann, R.W. Nitrous oxide (N₂O): The dominant ozone-depleting substance emitted in the 21st century. *Science* **2009**, *326*, 123–125. [[CrossRef](#)]
2. Myhre, G.; Shindell, D.; Bréon, F.-M.; Collins, W.; Fuglestedt, J.; Huang, J.; Koch, D.; Lamarque, J.-F.; Lee, D.; Mendoza, B.; et al. Anthropogenic and Natural Radiative Forcing. In *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*; Stocker, T.F., Qin, D., Plattner, G.K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M., Eds.; Cambridge University Press: Cambridge, UK; New York, NY, USA, 2013; pp. 659–740.
3. IPCC. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. In *Climate Change 2007: The Physical Science Basis*; Solomon, S., Qin, D., Manning, M., Marquis, M., Averyt, K., Tignor, M.M.B., Eds.; Cambridge University Press: Cambridge, UK, 2007.
4. Reay, D.S.; Davidson, E.A.; Smith, K.A.; Smith, P.; Melillo, J.M.; Dentener, F.; Crutzen, P.J. Global agriculture and nitrous oxide emissions. *Nat. Clim. Chang.* **2012**, *2*, 410. [[CrossRef](#)]
5. Maclean, J.; Hardy, B.; Hettel, G. *Rice Almanac: Source Book for One of the Most Important Economic Activities on Earth*; IRRI: Manila, Philippines, 2013.
6. Linquist, B.; Van Groenigen, K.J.; Adviento-Borbe, M.A.; Pittelkow, C.; Van Kessel, C. An agronomic assessment of greenhouse gas emissions from major cereal crops. *Glob. Chang. Biol.* **2012**, *18*, 194–209. [[CrossRef](#)]
7. Heffer, P. *Assessment of Fertilizer Use by Crop at the Global Level 2006–2007*; International Fertilizer Industry Association: Paris, France, 2009.
8. Zhao, X.; Pu, C.; Ma, S.T.; Liu, S.L.; Xue, J.F.; Wang, X.; Wang, Y.Q.; Li, S.S.; Lal, R.; Chen, F.; et al. Management-induced greenhouse gases emission mitigation in global rice production. *Sci. Total Environ.* **2019**, *649*, 1299–1306. [[CrossRef](#)] [[PubMed](#)]

9. Jiang, Y.; Carrijo, D.; Huang, S.; Chen, J.; Balaine, N.; Zhang, W.; Groenigen, K.J.; Linquist, B. Water management to mitigate the global warming potential of rice systems: A global meta-analysis. *Field Crop. Res.* **2019**, *234*, 47–54. [[CrossRef](#)]
10. Ussiri, D.; Lal, R. *Soil Emission of Nitrous Oxide and its Mitigation*; Springer: Dordrecht, The Netherlands, 2012.
11. Liu, X.; Zhou, T.; Liu, Y.; Zhang, X.; Li, L.; Pan, G. Effect of mid-season drainage on CH₄ and N₂O emission and grain yield in rice ecosystem: A meta-analysis. *Agric. Water Manag.* **2019**, *213*, 1028–1035. [[CrossRef](#)]
12. Yu, K.W.; Wang, Z.P.; Chen, G.X. Nitrous oxide and methane transport through rice plants. *Biol. Fert. Soils* **1997**, *24*, 341–343. [[CrossRef](#)]
13. Yan, X.Y.; Shi, S.L.; Du, L.J.; Xing, G. Pathways of N₂O emission from rice paddy soil. *Soil Biol. Biochem.* **2000**, *32*, 437–440. [[CrossRef](#)]
14. Lenhart, K.; Behrendt, T.; Greiner, S.; Steinkamp, J.; Well, R.; Giesemann, A.; Keppler, F. Nitrous oxide effluxes from plants as a potentially important source to the atmosphere. *New Phytol.* **2019**, *221*, 1398–1408. [[CrossRef](#)]
15. Smart, D.R.; Bloom, A.J. Wheat leaves emit nitrous oxide during nitrate assimilation. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 7875–7878. [[CrossRef](#)]
16. Goshima, N.; Mukai, T.; Suemori, M.; Takahashi, M.; Caboche, M.; Morikawa, H. Emission of nitrous oxide (N₂O) from transgenic tobacco expressing antisense NiR mRNA. *Plant J.* **1999**, *19*, 75–80. [[CrossRef](#)] [[PubMed](#)]
17. Lagomarsino, A.; Agnelli, A.E.; Linquist, B.; Adviento-Borbe, M.A.; Agnelli, A.; Gavina, G.; Ravaglia, S. and Ferrara, R.M. Alternate wetting and drying of rice reduced CH₄ emissions but triggered N₂O peaks in a clayey soil of central Italy. *Pedosphere* **2016**, *26*, 533–548. [[CrossRef](#)]
18. Kritee, K.; Nair, D.; Zavala-Araiza, D.; Proville, J.; Rudek, J.; Adhya, T.K.; Loecke, T.; Esteves, T.; Balireddygar, S.; Dava, O.; et al. High nitrous oxide fluxes from rice indicate the need to manage water for both long-and short-term climate impacts. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 9720–9725. [[CrossRef](#)] [[PubMed](#)]
19. Zou, J.; Huang, Y.; Zheng, X.; Wang, Y. Quantifying direct N₂O emissions in paddy fields during rice growing season in mainland China: Dependence on water regime. *Atmos. Environ.* **2007**, *41*, 8030–8042. [[CrossRef](#)]
20. Pittelkow, C.M.; Adviento-Borbe, M.A.; Hill, J.E.; Six, J.; van Kessel, C.; Linquist, B.A. Yield-scaled global warming potential of annual nitrous oxide and methane emissions from continuously flooded rice in response to nitrogen input. *Agric. Ecosyst. Environ.* **2013**, *177*, 10–20. [[CrossRef](#)]
21. Liu, H.; Ding, Y.; Zhang, Q.; Liu, X.; Xu, J.; Li, Y.; Di, H. Heterotrophic nitrification and denitrification are the main sources of nitrous oxide in two paddy soils. *Plant Soil* **2018**, *445*, 39–53. [[CrossRef](#)]
22. Hakata, M.; Takahashi, M.; Zumft, W.; Sakamoto, A.; Morikawa, H. Conversion of the nitrate nitrogen and nitrogen dioxide to nitrous oxides in plants. *Acta Biotechnol.* **2003**, *23*, 249–257. [[CrossRef](#)]
23. Dean, J.V.; Harper, J.E. Nitric oxide and nitrous oxide production by soybean and winged bean during the in vivo nitrate reductase assay. *Plant. Physiol.* **1986**, *82*, 718–723. [[CrossRef](#)]
24. Lenhart, K.; Weber, B.; Elbert, W.; Steinkamp, J.; Clough, T.; Crutzen, P.; Pöschl, U.; Keppler, F. Nitrous oxide and methane emissions from cryptogamic covers. *Glob. Chang. Biol.* **2015**, *21*, 3889–3900. [[CrossRef](#)]
25. Weathers, P.J. N₂O evolution by green algae. *Appl. Environ. Microbiol.* **1984**, *48*, 1251–1253. [[CrossRef](#)]
26. Brudvig, G.W.; Stevens, T.H.; Chan, S.I. Reactions of nitric oxide with cytochrome c oxidase. *Biochemistry* **1980**, *19*, 5275–5285. [[CrossRef](#)] [[PubMed](#)]
27. Rószter, T. *The Biology of Subcellular Nitric Oxide*; Springer: Dordrecht, The Netherlands, 2012.
28. Gupta, K.J.; Fernie, A.R.; Kaiser, W.M.; van Dongen, J.T. On the origins of nitric oxide. *Trends Plant. Sci.* **2011**, *16*, 160–168. [[CrossRef](#)] [[PubMed](#)]
29. Chamizo-Ampudia, A.; Sanz-Luque, E.; Llamas, A.; Galvan, A.; Fernandez, E. Nitrate reductase regulates plant nitric oxide homeostasis. *Trends Plant. Sci.* **2017**, *22*, 163–174. [[CrossRef](#)] [[PubMed](#)]
30. Gupta, K.J.; Igamberdiev, A.U. The anoxic plant mitochondrion as a nitrite: NO reductase. *Mitochondrion* **2011**, *11*, 537–543. [[CrossRef](#)] [[PubMed](#)]
31. Horchani, F.; Aschi-Smiti, S.; Brouquisse, R. Involvement of nitrate reduction in the tolerance of tomato (*Solanum lycopersicum* L.) plants to prolonged root hypoxia. *Acta Physiol. Plant.* **2010**, *32*, 1113–1123. [[CrossRef](#)]
32. Buwalda, F.; Greenway, H. Nitrogen uptake and growth of wheat during O₂ deficiency in root media containing NO₃⁻ only, or NO₃⁻ plus NH₄⁺. *New Phytol.* **1989**, *111*, 161–166. [[CrossRef](#)]

33. Zhang, B.G.; Puard, M.; Couchat, P. Effect of hypoxia, acidity and nitrate on inorganic nutrition in rice plants. *Plant Physiol. Biochem.* **1990**, *28*, 655–661.
34. Bailey-Serres, J.; Voeselek, L.A.C.J. Flooding stress: Acclimations and genetic diversity. *Annu. Rev. Plant Biol.* **2008**, *59*, 313–339. [[CrossRef](#)]
35. Oliveira, H.C.; Sodek, L. Effect of oxygen deficiency on nitrogen assimilation and amino acid metabolism of soybean root segments. *Amino Acids* **2013**, *44*, 743–755. [[CrossRef](#)]
36. Sugiura, M.; Georgescu, M.N.; Takahashi, M. A nitrite transporter associated with nitrite uptake by higher plant chloroplasts. *Plant Cell Physiol.* **2007**, *48*, 1022–1035. [[CrossRef](#)]
37. Stoimenova, M.; Igamberdiev, A.U.; Gupta, K.J.; Hill, R.D. Nitrite-driven anaerobic ATP synthesis in barley and rice root mitochondria. *Planta* **2007**, *226*, 465–474. [[CrossRef](#)] [[PubMed](#)]
38. Gupta, K.J.; Stoimenova, M.; Kaiser, W.M. In higher plants, only root mitochondria, but not leaf mitochondria reduce nitrite to NO, in vitro and in situ. *J. Exp. Bot.* **2005**, *56*, 2601–2609. [[CrossRef](#)] [[PubMed](#)]
39. Castello, P.R.; David, P.S.; McClure, T.; Crook, Z.; Poyton, R.O. Mitochondrial cytochrome oxidase produces nitric oxide under hypoxic conditions: Implications for oxygen sensing and hypoxic signaling in eukaryotes. *Cell Metab.* **2006**, *3*, 277–287. [[CrossRef](#)] [[PubMed](#)]
40. Jakobs, H.H.; Froriep, D.; Havemeyer, A.; Mendel, R.R.; Bittner, F.; Clement, B. The mitochondrial amidoxime reducing component (mARC): Involvement in metabolic reduction of N-oxides, oximes and N-hydroxyamido-hydrazones. *Chem. Med. Chem.* **2014**, *9*, 2381–2387. [[CrossRef](#)]
41. Sparacino-Watkins, C.E.; Tejero, J.; Sun, B.; Gauthier, M.C.; Thomas, J.; Ragireddy, V.; Merchant, B.A.; Wang, J.; Azarov, I.; Basu, P.; et al. Nitrite reductase and nitric-oxide synthase activity of the mitochondrial molybdopterins mARC1 and mARC2. *J. Biol. Chem.* **2014**, *289*, 10345–10358. [[CrossRef](#)]
42. Klepper, L.A. Nitric oxide emissions from soybean leaves during in vivo nitrate reductase assays. *Plant Physiol.* **1987**, *85*, 96–99. [[CrossRef](#)]
43. Rockel, P.; Strube, F.; Rockel, A.; Wildt, J.; Kaiser, W.M. Regulation of nitric oxide (NO) production by plant nitrate reductase in vivo and in vitro. *J. Exp. Bot.* **2002**, *53*, 103–110. [[CrossRef](#)]
44. Basu, S.; Azarova, N.A.; Font, M.D.; King, S.B.; Hogg, N.; Gladwin, M.T.; Surti, S.; Kim-Shapiro, D.B. Nitrite reductase activity of cytochrome c. *J. Biol. Inorg. Chem.* **2008**, *283*, 32590–32597. [[CrossRef](#)]
45. Benamar, A.; Rolletschek, H.; Borisjuk, L.; Avelange-Macherel, M.H.; Curien, G.; Mostefai, H.A.; Macherel, D. Nitrite–nitric oxide control of mitochondrial respiration at the frontier of anoxia. *BBA Bioenerg.* **2008**, *1777*, 1268–1275. [[CrossRef](#)]
46. Ascenzi, P.; Marino, M.; Polticelli, F.; Santucci, R.; Coletta, M. Cardiolipin modulates allosterically the nitrite reductase activity of horse heart cytochrome c. *J. Biol. Inorg. Chem.* **2014**, *19*, 1195–1201. [[CrossRef](#)]
47. Tischner, R.; Planchet, E.; Kaiser, W.M. Mitochondrial electron transport as a source for nitric oxide in the unicellular green alga *Chlorella sorokiniana*. *FEBS Lett.* **2004**, *576*, 151–155. [[CrossRef](#)] [[PubMed](#)]
48. Zhao, X.J.; Sampath, V.; Caughey, W.S. Cytochrome c oxidase catalysis of the reduction of nitric oxide to nitrous oxide. *Biochem. Biophys. Res. Commun.* **1995**, *212*, 1054–1060. [[CrossRef](#)]
49. Saraste, M.; Castresana, J. Cytochrome oxidase evolved by tinkering with denitrification enzymes. *FEBS Lett.* **1994**, *341*, 1–4. [[CrossRef](#)]
50. Blomberg, M.R.; Ädelroth, P. Mechanisms for enzymatic reduction of nitric oxide to nitrous oxide—A comparison between nitric oxide reductase and cytochrome c oxidase. *BBA Bioenerg.* **2018**, *1859*, 1223–1234. [[CrossRef](#)] [[PubMed](#)]
51. Alegria, A.E.; Sanchez, S.; Quintana, I. Quinone-enhanced ascorbate reduction of nitric oxide: Role of quinone redox potential. *Free Radic. Res.* **2004**, *38*, 1107–1112. [[CrossRef](#)] [[PubMed](#)]
52. Igamberdiev, A.U.; Hill, R.D. Plant mitochondrial function during anaerobiosis. *Ann. Bot.* **2008**, *103*, 259–268. [[CrossRef](#)] [[PubMed](#)]
53. Sanchez-Cruz, P.; Alegria, A.E. Quinone-enhanced reduction of nitric oxide by xanthine/xanthine oxidase. *Chem. Res. Toxicol.* **2009**, *22*, 818–823. [[CrossRef](#)]
54. Sauter, M. Root responses to flooding. *Curr. Opin. Plant Biol.* **2013**, *16*, 282–286. [[CrossRef](#)]
55. Winkel, A.; Colmer, T.D.; Ismail, A.M.; Pedersen, O. Internal aeration of paddy field rice during complete submergence—importance of light and floodwater O₂. *New Phytol.* **2013**, *197*, 1193–1203. [[CrossRef](#)] [[PubMed](#)]
56. Harper, J.E. Evolution of nitrogen oxide (s) during in vivo nitrate reductase assay of soybean leaves. *Plant Physiol.* **1981**, *68*, 1488–1493. [[CrossRef](#)] [[PubMed](#)]

57. Samuelson, M.E.; Öhlén, E.; Lind, M.; Larsson, C.M. Nitrate regulation of nitrate uptake and nitrate reductase expression in barley grown at different nitrate: Ammonium ratios at constant relative nitrogen addition rate. *Physiol. Plantarum*. **1995**, *94*, 254–260. [[CrossRef](#)]
58. Saravitz, C.H.; Devienne-Barret, F.; Raper, C.D., Jr.; Chaillou, S.; Lamaze, T. Nitrate uptake rate by soybean and wheat plants determined by external nitrate concentration and shoot-mediated demand. *Int. J. Plant Sci.* **1998**, *159*, 305–312. [[CrossRef](#)]
59. Kim, G.W.; Lee, J.G.; Gutierrez-Suson, J.; Khan, M.I.; Jung, S.T.; Kim, P.J. Importance of annual monitoring for evaluating the direct nitrous oxide emission factor in temperate mono-rice paddy fields. *Appl. Soil. Ecol.* **2019**, *140*, 42–48. [[CrossRef](#)]
60. Chaichana, N.; Bellingrath-Kimura, S.; Komiya, S.; Fujii, Y.; Noborio, K.; Dietrich, O.; Pakoktom, T. Comparison of Closed Chamber and Eddy Covariance Methods to Improve the Understanding of Methane Fluxes from Rice Paddy Fields in Japan. *Atmosphere* **2018**, *9*, 356. [[CrossRef](#)]
61. Swain, C.K.; Nayak, A.K.; Bhattacharyya, P.; Chatterjee, D.; Chatterjee, S.; Tripathi, R.; Dhal, B. Greenhouse gas emissions and energy exchange in wet and dry season rice: Eddy covariance-based approach. *Environ. Monit. Assess.* **2018**, *190*, 423. [[CrossRef](#)]
62. Machacova, K.; Vainio, E.; Urban, O.; Pihlatie, M. Seasonal dynamics of stem N₂O exchange follow the physiological activity of boreal trees. *Nat. Commun.* **2019**, *10*, 1–13. [[CrossRef](#)]
63. Decock, C.; Six, J. How reliable is the intramolecular distribution of ¹⁵N in N₂O to source partition N₂O emitted from soil? *Soil Biol. Biochem.* **2013**, *65*, 114–127. [[CrossRef](#)]
64. Firestone, M.K.; Firestone, R.B.; Tiedje, J.M. Nitrous oxide from soil denitrification: Factors controlling its biological production. *Science* **1980**, *208*, 749–751. [[CrossRef](#)]
65. Limami, A.M.; Diab, H.; Lothier, J. Nitrogen metabolism in plants under low oxygen stress. *Planta* **2014**, *239*, 531–541. [[CrossRef](#)] [[PubMed](#)]
66. Allègre, A.; Silvestre, J.; Morard, P.; Kallerhoff, J.; Pinelli, E. Nitrate reductase regulation in tomato roots by exogenous nitrate: A possible role in tolerance to long-term root anoxia. *J. Exp. Bot.* **2004**, *55*, 2625–2634. [[CrossRef](#)] [[PubMed](#)]
67. Shingaki-Wells, R.A.; Millar, A.H.; Whelan, J.; Narsai, R. What happens to plant mitochondria under low oxygen? An omics review of the responses to low oxygen and reoxygenation. *Plant Cell Environ.* **2014**, *37*, 2260–2277.
68. Gupta, K.J.; Mur, L.A.; Wany, A.; Kumari, A.; Fernie, A.R.; Ratcliffe, R.G. The role of nitrite and nitric oxide under low oxygen conditions in plants. *New Phytol.* **2019**, *225*, 1143–1151. [[CrossRef](#)] [[PubMed](#)]
69. Gupta, K.J.; Lee, C.P.; Ratcliffe, R.G. Nitrite protects mitochondrial structure and function under hypoxia. *Plant Cell Physiol.* **2016**, *58*, 175–183. [[CrossRef](#)] [[PubMed](#)]
70. Gupta, K.J.; Igamberdiev, A.U. Reactive nitrogen species in mitochondria and their implications in plant energy status and hypoxic stress tolerance. *Front. Plant Sci.* **2016**, *7*, 369. [[CrossRef](#)] [[PubMed](#)]
71. Igamberdiev, A.U.; Ratcliffe, R.G.; Gupta, K.J. Plant mitochondria: Source and target for nitric oxide. *Mitochondrion* **2014**, *19*, 329–333. [[CrossRef](#)] [[PubMed](#)]

