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HIGHLIGHT

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N-damo, an opportunity to reduce methane emissions?

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One of the greatest challenges in soil management is achieving balance between competing priorities. Compromise comes into play when complex goals exist such as increasing carbon sequestration, reducing greenhouse gas (GHG) emissions, minimizing nutrient loss and yielding high levels of profitable crops to feed a growing world population. This challenge is reflected in the increasing number of publications focused on how soil management practices affect the physico-chemical characteristics of soils, biodiversity and GHG emission (Huang et al., 2019; Ruan & Philip Robertson, 2013; Tamburini et al., 2020). Of these, some have investigated how replacing chemical fertilizers with biofertilizers affects these parameters (Park et al., 2022; Sessitsch et al., 2018; Vassileva et al., 2010). However, there is a lack of widely applicable and integrated predictors capable of determining the best management practices in the diverse agroecosystems found across the globe.

Of all sectors, agriculture and related land use emissions account for around 17% of global GHG emissions (FAO, 2018). Increased GHG concentrations in the atmosphere are the key contributor to climate change. Among these GHGs, methane (CH_4) is the second most important anthropogenic GHG for climate, after CO_2. The importance of CH_4 cannot be overstated. While CH_4 has a shorter half-life in the atmosphere than CO_2, it has around 30 times higher potential for affecting global warming. In addition, it is a precursor of important air pollutants such as ozone (Le Mer & Roger, 2001; Saunois et al., 2020). Rice paddies are a substantial source of CH_4, accounting for 8% of total global anthropogenic emissions. They also represent one of the most promising opportunities to mitigate these emissions (Kai et al., 2011; Saunois et al., 2020). To this end, increasing attention has been paid on unravelling the underlaying factors affecting CH_4 emission in this scenario (Shiau et al., 2018; Tang et al., 2019).

Methane-oxidizing bacteria can contribute substantially to the reduction of CH_4 emissions. It is estimated that aerobic methanotrophs can consume 40%–90% of total CH_4 emissions (Krüger et al., 2001; Le Mer & Roger, 2001). A group of bacteria recently discovered by Ettwig et al. (2010) called *Candidatus Methylomirabilis oxyfera* (*M. oxyfera*), triggers this process under the anaerobic conditions that are found in flooded rice paddies. These *M. oxyfera* bacteria are anaerobic methanotrophs that couple CH_4 oxidation to denitrification by using nitrite as an electron acceptor, linking the global carbon and nitrogen cycles (Ettwig et al., 2010). Interestingly, their genome includes all the genes necessary for aerobic CH_4 oxidation. The proposed pathway for nitrite-dependent anaerobic methane oxidation (n-damo) involves the reduction of nitrite to dinitrogen with oxygen production as a metabolic intermediate used for CH_4 oxidation to CO_2 (Figure 1) (Ettwig et al., 2010). Because nitrogen oxides were already present on the early Earth, this discovery is of evolutionary interest because it makes possible that oxygen became available to microbial metabolism prior to the evolution of oxygenic photosynthesis (Ettwig et al., 2010). Although there is increasing interest in this process, the quantitative contribution of the n-damo

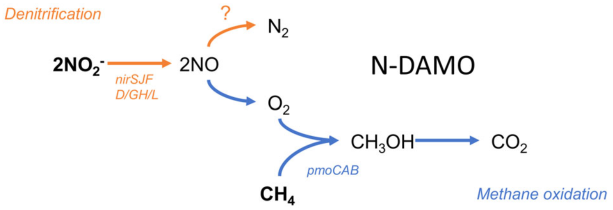

FIGURE 1 Proposed pathway for n-damo process. Scheme based on Ettwig et al. (2010) with the corresponding enzymes identified by the authors in *Candidatus Methylomirabilis oxyfera* genome: *nirSJFD*/*GH*/*L*, nitrite reductase; *pmoCAB*, particulate methane monooxygenase; ?, unknown enzyme

process to mitigate CH_4 emissions from paddy ecosystems and how management soil practices can alter this process are poorly understood (Shen et al., 2014, 2021; Vaksmaa et al., 2016).

One of the keys, and most widespread practices, to obtain high-quality crops is the use of chemical and organic fertilizers. At the same time, a sustained fertilization over time can dramatically impact the physicochemical characteristics of soils (i.e. pH, moisture, total carbon and nitrogen) as well as microbial abundance and composition, altering nutrient fluxes, gas emissions and the entire agroecosystem dynamics (Huang et al., 2019; Hui et al., 2017; Shen et al., 2021).

The excessive use of fertilizers has led to serious environmental problems such as eutrophication due to nitrogen leaching into the water table. Thus, a better understanding of how fertilization affects the n-damo process could provide novel strategies to improve fertilizer and soil management, reduce nitrogen loss and regulate CH_4 anthropogenic emissions. Recently, Shen et al. (2021) conducted a 5-year experiment in which the team applied chemical and organic fertilizers (i.e., urea and chicken manure) to rice paddy fields and found that this combination successfully stimulated *M. oxyfera* activity. Long-term experiments like this are key to unlocking improved soil management practices capable of increasing and sustaining *M. oxyfera* populations.

Another study published in *Environmental Microbiology* by Yang et al. (2022) investigated the effect of three long-term (32 years) fertilization treatments on the potential activity, abundance and community composition of nitrite-dependent anaerobic methanotrophs in paddy fields. The three conditions used were: (i) unfertilized control, (ii) chemical fertilization and (iii) chemical fertilization with straw incorporation. Given the duration of the experiment and that soil samples were taken over a wide range of soil depths and rice growth stages, this study serves as a reliable benchmark on the effect of nitrogen on n-damo processes. Their results reveal that both fertilization treatments increased n-damo activity as well as the abundance of *M. oxyfera*-like bacteria compared to the unfertilized control, with the greatest gains seen when chemical and organic fertilization were combined. However, no significant differences were found in the community composition or the diversity of *M. oxyfera*-like bacteria between conditions, and they reported that only soil depth seemed to affect these properties. The dynamics of n-damo activity and abundance of *M. oxyfera*-like bacteria were closely correlated with changes in physicochemical characteristics of soils likely induced by fertilizer use (i.e., pH, soil organic carbon and water content, NH_4^+, NO_2^- and NO_3^-). Furthermore, their findings revealed that the n-damo process was especially active in the upper part of the soil (0–10 cm). Also, soil organic content and inorganic forms of nitrogen had a clearly positive effect on the n-damo process, likely because of the increase in organic substrate sources and increased NO_2^- concentrations (i.e., which serves as the electron acceptor in the process). Taken together, these results highlight the potential that *M. oxyfera*-like bacteria have for the mitigation of CH_4 emissions from paddy fields under long-term fertilization. However, in this study, CH_4 emissions were not measured. Therefore, currently, a direct link between long-term fertilization, the n-damo process and CH_4 emissions cannot be established. The uncertainty between these factors is made even more apparent by other studies, which found that fertilizer use increased CH_4 emissions from paddy fields and that this may be caused by the enhanced activity of methanogens (Wang et al., 2020; Zhang et al., 2015). Thus, the stimulation of n-damo activity might not be enough to cope with the increased CH_4 production. An important approach that is needed to unravel this mystery is the use of soil microbiome analysis. Studying *M. oxyfera*-like bacteria, in addition to community markers involved in the carbon and nitrogen cycles would offer a view of both cycles and provide a clearer picture of the key players driving n-damo activity.

Overall, the authors have provided important insights into the effect of sustained fertilization on the n-damo process. However, experimental models that incorporate the measurement of direct CH_4 levels are needed to underpin the role of the n-damo process in mitigating emissions and fine-tuning the use of fertilizers in rice cultivation. In addition, future studies with even broader scopes are needed to test the effect of other aspects of management practices—such as the effect of bacteria supplementation. It is vital that we answer these questions. Restoring balance between methanogens and methanotrophs in this scenario could lead to practical approaches to reduce CH_4 emissions world-wide—and, ultimately, serve as a powerful tool in the fight against climate change.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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