Nitrous oxide (N₂O) synthesis by *Microcystis aeruginosa*

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**Abstract.** Pure cultures of *Microcystis aeruginosa* synthesized nitrous oxide (N₂O) when supplied with nitrite (NO₂⁻) in darkness (198.9 nmol·g-DW⁻¹·h⁻¹ after 24 hours) and illumination (163.1 nmol·g-DW⁻¹·h⁻¹ after 24 hours) whereas N₂O production was negligible in abiotic controls supplied with NO₂⁻ and in cultures deprived of exogenous nitrogen. N₂O production was also positively correlated to the initial NO₂⁻ and *M. aeruginosa* concentrations, but low to negligible when nitrate (NO₃⁻) and ammonium (NH₄⁺) were supplied as the sole exogenous N source instead of NO₂⁻. A protein database search revealed *M. aeruginosa* possesses protein homologues to eukaryotic microalgae enzymes known to catalyse the successive reduction of NO₂⁻ into nitric oxide (NO) and N₂O. Our laboratory study is the first demonstration that *M. aeruginosa* possesses the ability to synthesize N₂O. As *M. aeruginosa* is a bloom-forming cyanobacterium found globally, further research (including field monitoring) is now needed to establish the significance of N₂O synthesis by *M. aeruginosa* under relevant conditions (especially in terms of N supply). Further work is also needed to confirm the biochemical pathway and potential function of this synthesis.

**Graphical Abstract.**

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1
Introduction

Emissions of the potent ozone depleting greenhouse gas nitrous oxide (N\textsubscript{2}O) have been reported from various aquatic ecosystems characterized by a high level of photosynthetic activity and several authors have suggested that N\textsubscript{2}O emissions from eutrophic lakes could be globally significant (Delsontro et al., 2018; Plouviez et al., 2019a). Noteworthy, Delsontro et al. (2018) determined that N\textsubscript{2}O emissions from lakes and impoundments could be expected to increase as a function of lake size and chlorophyll a (an indicator of the presence of primary producer such as microalgae). Because eutrophication is an increasing global issue (Delsontro et al., 2018; Kapsalis and Kalavrouziotis, 2021; Maure et al., 2021), N\textsubscript{2}O emissions from these ecosystems could also be expected to increase. Several species of microalgae and cyanobacteria can indeed synthesize N\textsubscript{2}O (Weathers, 1984; Weathers and Niedzielski, 1986; Bauer et al., 2016; Plouviez et al., 2019a) and a biochemical pathway for this synthesis has been established in the model microalga Chlamydomonas reinhardtii (Plouviez et al., 2017b; Burlacot et al., 2020). Despite these critical advances, the true global significance of microalgal N\textsubscript{2}O synthesis in microalgae-rich eutrophic aquatic bodies remains unknown (Plouviez et al., 2019a; Burlacot et al., 2020; Plouviez and Guieysse, 2020). Microcystis species are cyanobacteria commonly found in eutrophic ecosystems (Xiao et al., 2018; Zhou et al., 2020; Hernandez-Zamora et al., 2021) but the ability of this genus to synthesize N\textsubscript{2}O is currently unknown. We, therefore, investigated the ability of N\textsubscript{2}O production by the most notorious bloom-forming cyanobacterium reported in freshwaters and model cyanobacterium M. aeruginosa (Qian et al., 2010; Kataoka et al., 2020; Zhou et al., 2020) under conditions known to induce or impact N\textsubscript{2}O production in microalgae (Guieysse et al., 2013; Alcantara et al., 2015; Bauer et al., 2016; Plouviez et al., 2017b; Burlacot et al., 2020).

Results and discussion

2.1 N\textsubscript{2}O synthesis bioassays

The ability of M. aeruginosa to synthesize N\textsubscript{2}O was investigated using a protocol successfully used for the microalgae C. vulgaris and C. reinhardtii (Alcantara et al., 2015; Guieysse et al., 2013; Plouviez et al., 2017b). As can be seen in Fig. 1, N\textsubscript{2}O production was only recorded in cultures supplied NO\textsubscript{2}\textsuperscript{-} as there was no significant production in the absence of the cyanobacterium (abiotic control) or the absence of NO\textsubscript{2}\textsuperscript{-} (negative control). Further assays showed a positive correlation between biomass concentration and N\textsubscript{2}O production (Fig. 2), confirming the biological origin of N\textsubscript{2}O synthesis.
Figure 1. Total N₂O accumulation (nmole) from *M. aeruginosa* supplied with 10 mM NO₂⁻ under continuous illumination (○, n ≥ 12), abiotic control N-free media with 10 mM NO₂⁻ (+, n ≥ 10) and negative control: *M. aeruginosa* cultures incubated in N-free media (Δ, n ≥ 10).
Figure 2. Normalized N₂O production (nmol·g-DW⁻¹) recorded from *M. aeruginosa* cultures with different biomass concentrations (0.1, 0.2 and 0.4 g-DW·L⁻¹; n ≥ 6, n ≥ 12, n = 4, respectively) in sealed flasks supplied light and 10 mM NO₂⁻. N₂O synthesis was statistically different when comparing the rates between 2.5 and 24 h and between 24 and 48 h (p < 0.05, two samples t-test). Specific N₂O production rates (nmol N₂O·g DW⁻¹·h⁻¹) can be found in S2.

In comparison to cultures supplied with NO₂⁻, low and negligible N₂O synthesis was recorded in cultures supplied with NO₃⁻ and NH₄⁺, respectively (Table 1). This showed that NO₂⁻ was the substrate to N₂O synthesis, as reported for other microalgae (Weathers, 1984; Weathers and Niedzielski, 1986; Guieysse et al., 2013; Alcantara et al., 2015; Bauer et al., 2016; Plouviez et al., 2017b; Burlacot et al., 2020). This was also confirmed by the positive correlation between NO₂⁻ concentration and N₂O synthesis (S3, Vmax = 185 nmol·g-DW⁻¹·h⁻¹ and Km for NO₂⁻ = 2.22 mM).

Table 1. N₂O emissions in different conditions (n = number of replicates)

<table>
<thead>
<tr>
<th>Light conditions</th>
<th>N source</th>
<th>N₂O production (nmol·g-DW⁻¹·h⁻¹)</th>
<th>Standard error</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light</td>
<td>1 mM NO₂⁻</td>
<td>59.5</td>
<td>13.7</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>5 mM NO₂⁻</td>
<td>131.5</td>
<td>21.5</td>
<td>16</td>
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<tr>
<td></td>
<td>10 mM NO₂⁻</td>
<td>163.1</td>
<td>31.5</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>10 mM NO₃⁻</td>
<td>3.9</td>
<td>1.4</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>10 mM NH₄⁺</td>
<td>0.07</td>
<td>0.7</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>0.9</td>
<td>0.5</td>
<td>4</td>
</tr>
<tr>
<td>Dark</td>
<td>10 mM NO₂⁻</td>
<td>198.9</td>
<td>30.5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>1.5</td>
<td>1.7</td>
<td>6</td>
</tr>
</tbody>
</table>

*M. aeruginosa* was able to synthesize N₂O in both darkness and illumination (Table 1), respectively representing 0.07% and 0.06% of the amount of N supplied (g-N-N₂O produced/g-N supplied × 100). The N₂O produced under illumination was statistically lower than in darkness (p-value < 0.05, two samples t-test, n = 5 replicates from experiments performed on the same day). The negative impact of light was previously observed in *C. vulgaris* and *C. reinhardtii* tested under similar conditions (Guieysse et al., 2013; Alcantara et al., 2015; Plouviez et al., 2017b), although N₂O production was positively correlated with light supply in *C. vulgaris* grown outdoors (Plouviez et al., 2017a). The difference we observed during this study may be explained by light-dependent mechanisms impacting enzymatic activities and consequently intracellular NO₂⁻ accumulation (e.g. the rates of NO₂⁻ reduction into NH₄⁺ and N₂O), as suggested by Plouviez et al. (2017a). However, O₂ production during photosynthesis could also influence N₂O synthesis. Burlacot et al. (2020) indeed reported that one of the enzymes involved in NO reduction to N₂O (Flavodiiron, as discussed in the next section) can also catalyse the reduction of O₂.
into H_2O. Because of this dual activity and the reactivity of NO with O_2, N_2O production could be sensitive to O_2. Further research is therefore needed to understand if O_2 influence N_2O production by competitive NO conversion to products such as nitrogen oxides and peroxynitrite, or/and by competitive O_2 reduction into H_2O instead of its reduction to N_2O by the enzymes with nitric reductase ability.

While small, N_2O synthesis was statistically significant in *M. aeruginosa* fed NO_3^- as the sole exogenous N source (p-value < 0.05, two samples t-test when compared with the negative controls). As in *C. vulgaris* and *C. reinhardtii*, the intracellular reduction of NO_3^- into NO_2^- by the enzyme nitrate reductase (narB) is the first step of NO_3^- assimilation in *M. aeruginosa* (Ohashi et al., 2011; Zhou et al., 2020). Hence, intracellular NO_2^- production likely generated this substrate for N_2O synthesis during NO_3^- exogenous supply but competitive use of NO_2^- (for protein synthesis via NH_4^+ generation) could have competed with N_2O synthesis. Intracellular NO_2^- production and accumulation is not expected when cells assimilate NH_4^+ (Plouviez et al., 2019), explaining the absence of N_2O production in the flasks supplied NH_4^+ as sole exogenous N source (p-value = 0.91, two samples t-test when compared with the negative controls). In *M. aeruginosa*, NO_3^- uptake and the transcriptional regulation of nitrate reductase have been shown to be activated by light, NO_3^- and NO_2^- (Chen et al., 2009; Ohashi et al., 2011; Chen and Liu, 2015). While the transcriptional and post-translational regulation of nitrate reductase in *M. aeruginosa* still needs to be investigated in relation to N_2O synthesis and varying environmental parameters (e.g. light supply), it is possible that the pattern of N_2O synthesis during outdoor *M. aeruginosa* growth would be similar to that seen in *C. vulgaris*.

### 2.2 Putative pathways

In the eukaryotic microalga *C. reinhardtii*, cytoplasmic NO_2^- is sequentially reduced to nitric oxide (NO) and N_2O. The first step, NO_2^- reduction into NO, is catalysed by the dual enzyme nitrate reductase-NO forming nitrite reductase (NR-NoFNiR) or, potentially, the copper containing nitrite reductase (NirK). The second step, NO reduction into N_2O, can then be catalysed by cytochrome P450 (CYP55, Plouviez et al., 2017b; Burlacot et al., 2020), Flavodiirons (FLVs, Burlacot et al., 2020; Bellido-Pedraza et al., 2020), or potentially by the Hybrid Cluster proteins (HCPs, Bellido-Pedraza et al., 2020) involved in nitrogen metabolism (Van Lis et al., 2020). Interestingly, NO_2^- reduction into NO by nitrate reductase (narB) has been demonstrated in *M. aeruginosa* (Tang et al., 2011; Song et al., 2017) and here we found that *M. aeruginosa* possesses homologs of the CYP55, FLVs, and HCPs found in *C. reinhardtii* ([Table. 2](#)). While the functions of these proteins need to be confirmed, their presence suggests N_2O synthesis in *M. aeruginosa* could involve similar NO_2^- and NO reduction pathways to those found in *C. reinhardtii*.

<table>
<thead>
<tr>
<th>Accession number</th>
<th>Description</th>
<th>Blastp results</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP55</td>
<td>100% identity</td>
<td></td>
</tr>
<tr>
<td>Flavodiirons</td>
<td>85% identity</td>
<td></td>
</tr>
<tr>
<td>Hybrid Cluster proteins</td>
<td>70% identity</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Summary of Blastp results for proteins potentially involved in N_2O synthesis in *Chlamydomonas reinhardtii*. Accession numbers were retrieved from (Bellido-Pedraza et al., 2020) and used as query sequence for blastp (protein-protein BLAST) protein searches (https://blast.ncbi.nlm.nih.gov/Blast.cgi) of *M. aeruginosa* (taxid:1126) protein sequences database.
### Table

<table>
<thead>
<tr>
<th>Protein</th>
<th>C. reinhardtii accession number</th>
<th>e-value</th>
<th>M. aeruginosa accession number</th>
<th>% Similarity</th>
<th>M. aeruginosa protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>NirK</td>
<td>PNW79625.1</td>
<td>-</td>
<td>NCR75269.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HCP</td>
<td>XP_001694756.1</td>
<td>3e-158</td>
<td>NCR75269.1</td>
<td>45.38</td>
<td>Hydroxylamine reductase</td>
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<td>XP_001694571.1</td>
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<td>XP_001694671.1</td>
<td>2e-157</td>
<td>NCR75269.1</td>
<td>45.03</td>
<td>Hydroxylamine reductase</td>
</tr>
<tr>
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<td>XP_001694454.1</td>
<td>2e-159</td>
<td>WP_002787796.1</td>
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<td>FLV</td>
<td>XP_001692916.1</td>
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<td>FLV</td>
<td>PNW71243.1</td>
<td>0</td>
<td>WP_11054956.1</td>
<td>52.18</td>
<td>Diflavin flavoprotein</td>
</tr>
</tbody>
</table>

### 2.3 Metabolic function

The metabolic function of N₂O synthesis in eukaryotic microalgae is currently unknown and it has been suggested that NO₂⁻ reduction into N₂O enables cells to expend excess energy or instead, is the fortuitous result of dual enzymatic activity (Guieysse et al., 2013; Plouviez et al., 2017b). The intermediate NO is a ubiquitous signalling molecule in algae (Astier et al., 2021). Interestingly, NO stimulates the production of secondary metabolites (e.g. linoleic acid) by *M. aeruginosa* that inhibit the growth of competitors (Song et al., 2017). NO also promotes the growth of this cyanobacterium (Tang et al., 2011). While the link between NO and N₂O still needs to be determined, it is possible that the NO and N₂O biosynthetic pathways is/are involved in cell-to-cell communications in *M. aeruginosa* and more broadly, in microalgae. Further research is needed.

### 2.4 Potential environmental implications

Microalgae species from at least 3 divisions (Bacillariophyta, Chlorophyta, Cyanobacteria) have the ability to synthesize NO (Kim et al., 2008; Kumar et al., 2015; Plouviez et al., 2017b; Tang et al., 2011) and/or N₂O (Weathers, 1984; Weathers and Niedzielski, 1986; Guieysse et al., 2013; Kamp et al., 2013; Plouviez et al., 2017a, b, this study). All these observations suggest that the ability to synthesize N₂O is widely distributed among microalgae. Critically, N₂O emissions from aquatic environments where microalgae abound, such as oceans, lakes and engineered cultivation systems, have been repeatedly reported (Bauer et al., 2016; Plouviez et al., 2019b; Plouviez et al., 2019a; Zhang et al., 2022) even under very low exogenous NO₂⁻ concentrations (Plouviez et al., 2019b). These emissions can be explained by intracellular NO₂⁻ production during reductive nitrate assimilation (Plouviez et al., 2017a, b, 2019b) under conditions when excess NO₂⁻ production (Bristow et al., 2015; French et al., 1983; Mortonson and Brooks, 1980; Schaefer and Hollibaugh, 2018) could support N₂O synthesis.
Based on the data available, DelSontro et al. (2018) and Plouviez and Guieysse, (2020) estimated that global \( \text{N}_2\text{O} \) emissions from eutrophic lakes alone could represent 110 to 450 kt \( \text{N}_2\text{O} \cdot \text{yr}^{-1} \), which represent 14-56\% of the natural and anthropogenic \( \text{N}_2\text{O} \) emissions reported from inland and coastal waters (Tian et al., 2020). Importantly, DelSontro et al. (2018) predicted that \( \text{N}_2\text{O} \) emissions from lakes and impoundments would increase with lake size and chlorophyll a concentration. The \( \text{N}_2\text{O} \) synthesis rates reported during our study are in the same order of magnitude as the rate previously reported for members of the green microalgae, cyanobacteria, and diatoms (Bauer et al., 2016; Plouviez et al., 2019a). However, we cannot conclude that \( M. \text{ aeruginosa} \) (or other species) is or is not a major \( \text{N}_2\text{O} \) producer in lakes and other aquatic environments without evidence from field measurements. Indeed, high \( \text{NO}_2^- \) concentrations are rare in natural and engineered ecosystems environments, which would suggest insignificant microalgal \( \text{N}_2\text{O} \) production in most contexts. Nevertheless, significant \( \text{N}_2\text{O} \) emissions were reported from outdoor cultures of \( C. \text{ vulgaris} \) fed \( \text{NO}_3^- \) (Guieysse et al., 2013; Plouviez et al., 2017), despite this alga also producing much more \( \text{N}_2\text{O} \) when fed \( \text{NO}_2^- \) (Guieysse et al., 2013). Plouviez et al. (2017) suggested this was caused by \( \text{NO}_2^- \) intracellular accumulation under varying light, as this condition is known to have different impacts on the rate of \( \text{NO}_3^- \) reduction into \( \text{NO}_2^- \) by NR and the rate of \( \text{NO}_2^- \) reduction into \( \text{NH}_4^+ \) by NiR. During our study, \( \text{N}_2\text{O} \) emissions under \( \text{NO}_3^- \) supply were low, but not negligible. Because NR activity is also influenced by light and the availabilities of \( \text{NO}_3^- \) and \( \text{NO}_2^- \) in \( M. \text{ aeruginosa} \) (Chen et al., 2009; Ohashi et al., 2011; Chen and Liu, 2015), \( \text{N}_2\text{O} \) synthesis by this microalga could possibly occur in environments where \( \text{NO}_3^- \) is the main nitrogen source.

Our findings support past predictions of the global relevance of photosynthetic \( \text{N}_2\text{O} \) emissions from eutrophic aquatic bodies as Microcystis is globally found and often the dominant genus in these ecosystems (Qian et al., 2010; Kataoka et al., 2020; Zhou et al., 2020). The work from Weathers and Niedzielski, (1986) and ours suggest that \( Nostoc \) spp., \( Aphanocapsa \) (PCC 6308), \( Aphanocapsa \) (PCC 6714) and \( M. \text{ aeruginosa} \) have the ability to synthesize \( \text{N}_2\text{O} \). Consequently, other cyanobacteria species may also have this ability. Further research is now needed to quantify \( \text{N}_2\text{O} \) emissions from eutrophic aquatic ecosystems where cyanobacteria abound. This is especially timely considering that the frequency and geographic distribution of harmful algae blooms have increased due to anthropogenic activities (Paerl et al., 2018; Kataoka et al., 2020). In addition, algae blooms can lead to the decrease of \( \text{O}_2 \) in oceans, coastal waters and lakes (Jenny et al., 2015; Rabalais and Turner, 2019), a condition that can increase the accumulation of \( \text{NO}_2^- \) in aquatic ecosystems (Schaefer and Hollibaugh, 2018; Bristow et al., 2015). Because microalgal \( \text{N}_2\text{O} \) synthesis is rapid and influenced by factors such as the cell biology (Plouviez et al., 2019b) and, as observed during our study, the type and concentration of the nitrogen source microalgae receive, extensive monitoring (i.e. long-term with wide spatial coverage and high sampling frequency) of several types of microalgae-rich environments are required (e.g. hypoxic waters).
3 Conclusions

We herein present the first demonstration that *M. aeruginosa* synthesizes N₂O. *Microcystis aeruginosa* synthesized N₂O when supplied with NO₂⁻ in darkness (198.9 nmol·g-DW⁻¹·h⁻¹ after 24 hours) and illumination (163.1 nmol·g-DW⁻¹·h⁻¹ after 24 hours), and this production was positively correlated to the initial NO₂⁻ and *M. aeruginosa* concentrations. A protein database search also revealed *M. aeruginosa* possesses proteins homologues to eukaryotic microalgae known to catalyse the successive reduction of NO₂⁻ into NO and N₂O. As *M. aeruginosa* is globally distributed, further research (including field monitoring) is now needed to evaluate the significance of N₂O synthesis by these cyanobacteria under relevant conditions (especially in terms of N supply). Further studies are also needed to confirm the genes/proteins involved. Further studies are needed to confirm the genes/proteins involved as a better understanding of the biochemical pathway involved during microalgal N₂O synthesis is critical to efficiently monitor (i.e. identify the source) and mitigate N₂O emissions.

Our study is another evidence of the ability of photosynthetic microorganisms, especially cyanobacteria, to synthesize N₂O. Preliminary estimation showed that N₂O emissions from eutrophic lakes alone could represent 110 to 450 kt N-N₂O·yr⁻¹, which represent 14-56% of the natural and anthropogenic N₂O emissions reported from inland and coastal waters. However, how much microalgae contribute to these emissions is currently unknown. As *M. aeruginosa* is globally distributed, further research (including field monitoring with wide spatial coverage, high sampling frequency and water type) is now needed to evaluate the significance of N₂O synthesis by these cyanobacteria under relevant conditions (especially in terms of N supply).

4 Appendix: Materials and Methods

4.1 Strain and culture maintenance

*Microcystis aeruginosa* UTEX 2385 was obtained from the culture collection of the University of Texas at Austin (https://utex.org/). Pure cultures were maintained on 100 mL low-phosphate minimal media (Plouviez et al., 2021) incubated at 25°C (INFORS HT Multitron) under continuous illumination (20 µmol·cm⁻²·s⁻¹) and agitation (150 rotation per minutes, rpm). Cultures thus incubated for more than a week were supplied with 100 µL of a solution of KH₂PO₄/K₂HPO₄ (0.4 M/0.6 M) to prevent P limitation. The purity of the cultures was verified via sequencing (S1).

4.2 Cultivation and Bioassays

*M. aeruginosa* was cultivated on 400 mL low-phosphorus minimal media in 500 mL Duran bottles for 5 days. These cultures were incubated under fluorescent tubes (F15W/GRO sylvania gro-lux) providing illumination at 20 µmol·cm⁻²·s⁻¹ at the culture surface. Mixing was provided by bubbling filtered (0.22µm) air at 1.5 L·min⁻¹. On the day of the experiment, 15 mL aliquots
were withdrawn from the cultures to measure the cell dry weight (DW) according to (Bechet et al., 2015). Then, 100-400 mL aliquots were centrifuged at 4400 rpm for 3 min. The supernatants were discarded, and the pellets were re-suspended in N-free medium to a final concentration of 0.2 g-DW L⁻¹ as previously described (Guieysse et al., 2013). Twenty-five mL aliquots of these suspensions were transferred into 120 mL serum flasks supplied with 1 mL of NaNO₂, NaNO₃ or NH₄Cl stock solutions (250 mM) to reach a final concentration of 10 mM. Sterile abiotic controls were not inoculated but were supplied with 10 mM nitrite (NO₂⁻) while negative controls were *M. aeruginosa* cultures incubated in N-free media. The flasks were immediately sealed with rubber septa and aluminium caps and incubated at 25°C under continuous agitation (150 rpm) under either constant illumination (20 µmol·cm⁻²·s⁻¹) or darkness. A similar protocol was used to evaluate the impact of the initial cell (0.1 – 0.4 g-DW·L⁻¹), NO₂⁻ (1 – 10 mM) or nitrate (NO₃⁻, 10 mM) or ammonium (NH₄⁺, 10 mM) concentrations on N₂O synthesis. Unless otherwise stated, each condition was tested in triplicate flasks and repeated at least twice. All glassware and media were autoclaved prior to the experiments. An additional experiment confirmed the purity of the *M. aeruginosa* stock cultures and the cultures used during the bioassays (S1).

4.3 Analysis

Gas samples (5 mL) were withdrawn from the flask headspace using a syringe equipped with a needle. The headspace N₂O concentration in those samples was then quantified using gas chromatography (Shimadzu GC-2010, Shimadzu, Japan). Total N₂O was calculated as the sum of gaseous N₂O and dissolved N₂O as described by Guieysse et al. (2013). Briefly, Assuming the gas and the liquid phase N₂O concentrations were at equilibrium at the time of sampling, the total amount of N₂O produced in the flask was calculated by summing up the amounts of N₂O present in the gas and liquid phases. The amount of dissolved N₂O in the liquid phase was calculated using Henry’s law at 25°C (Eq. 1):

\[
n_{N_2O_{\text{total}}}^t = x_{N_2O}^t \cdot P^t \cdot \left( \frac{V_g}{R \cdot T} + H_{N_2O} \cdot V_l \right)
\]

Where \(n_{N_2O_{\text{total}}}^t\) is the total amount of N₂O produced in the Duran bottle at time t (moles N₂O); \(x_{N_2O}^t\) is the molar fraction of N₂O in the gas phase at time t (mol N₂O·mol gas⁻¹); \(P^t\) is the pressure in the gas headspace at time t (typically 101325 Pa unless otherwise stated); \(V_g\) is the volume of gas in the flask (mL); \(R\) is the ideal gas constant (8.314 J·mol⁻¹·K⁻¹); \(T\) is the temperature inside the bottle (298.15 K); \(H_{N_2O}\) is the Henry law constant of N₂O at T (2.5·10⁻⁷ mol·L⁻¹·Pa⁻¹); and \(V_l\) is the volume of liquid in the serum flask (mL).

Supplementary information

The purity of *M. aeruginosa* cultures was confirmed by PCR and sequencing. The methodology used and the results obtained are presented in the supplement S1.
Authors contribution

F.F. performed the investigation, data visualization and curation, and contributed to the writing - review & editing of the manuscript. M.P. was involved with the writing - original draft and contributed to conceptualization, methodology, and data curation and visualization with B.J. B.J. and J.P. were involved with the writing - review & editing of the manuscript before submission. Finally, B.J., J.P. and M.P. were all involved with the funding acquisition.

Competing interests

The authors declare that they have no conflict of interest.

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References


