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Algal photosynthesis converts nitric oxide into nitrous oxide

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Nitrous oxide (N₂O), a potent greenhouse gas in the atmosphere, is produced mostly from aquatic ecosystems, to which algae substantially contribute. However, mechanisms of N₂O production by photosynthetic organisms are poorly described. Here we show that the green microalga *Chlamydomonas reinhardtii* reduces NO into N₂O using the photosynthetic electron transport. Through the study of *C. reinhardtii* mutants deficient in flavodiiron proteins (FLVs) or in a cytochrome p450 (CYP55), we show that FLVs contribute to NO reduction in the light, while CYP55 operates in the dark. Both pathways are active when NO is produced in vivo during the reduction of nitrites and participate in NO homeostasis. Furthermore, NO reduction by both pathways is restricted to chlorophytes, organisms particularly abundant in ocean N₂O-producing hot spots. Our results provide a mechanistic understanding of N₂O production in eukaryotic phototrophs and represent an important step toward a comprehensive assessment of greenhouse gas emission by aquatic ecosystems.

microalgae | nitrous oxide | photosynthesis | flavodiiron | cytochrome P450

Although nitrous oxide (N₂O) is present in the atmosphere at concentrations 1,000 times lower than CO₂, it is recognized as the third most potent greenhouse gas after CO₂ and CH₄, contributing to ~6% of the total radiative forcing on Earth (1, 2). In addition, N₂O is the main ozone-depleting gas produced in our planet (3). Since 1970, N₂O concentration in the atmosphere has been rising, reaching the highest measured production rate in the past 22,000 y (2). Natural sources of N₂O account for 64% of the global N₂O production, mostly originating from soils and oceans (4). Bacteria and fungi widely contribute to this production, N₂O being produced during nitrification by the reduction of nitric oxide (NO) mediated by NO reductases (NORs) (5, 6).

Microalgae are primary biomass producers in oceans and lakes. For decades, N₂O production has been detected in samples from the ocean, lakes, and coastal waters, but the respective contribution of prokaryotic and eukaryotic organisms to this phenomenon remains to be investigated (7–9), and particularly the contribution of microalgae has been overlooked (7, 10). Algal blooms correlate with N₂O production (8, 10, 11), and recently, axenic microalgal cultures were shown to produce a substantial amount of N₂O (12–14). Despite the possible ecological significance of microalgae in N₂O emissions (10), little is known about the molecular mechanisms of N₂O production in microalgae.

In bacteria, membrane-bound NORs belong to the haem/copper cytochrome oxidase family (15), some of which containing a c-type cytochrome (16). Soluble bacterial NORs belong to the flavodiiron family (FLVs), enzymes able to reduce NO and/or O₂ (17). In fungi, NORs are soluble and belong to the cytochrome P450 (CYP55) family (18, 19). The genome of the model green microalga *Chlamydomonas reinhardtii* harbors both a CYP55 fungal homolog (12) and FLVs bacterial homologs (20). Based on ribonucleic acid interference (RNAi) silencing experiments in *C. reinhardtii*, Plouviez et al. (13) concluded that the CYP55 homolog is the main contributor to N₂O production. However, this gene has so far only been identified in three sequenced algal genomes (21), suggesting the occurrence of other mechanisms. *C. reinhardtii* FLVs have been recently shown to be involved in O₂ photoreduction (22), but have not been considered as catalyzing NO

reduction so far (23). Thus, the major players involved in N₂O production in microalgae remain to be elucidated.

In this work, by measuring NO and N₂O gas exchange using a membrane inlet mass spectrometer (MIMS) during dark to light transitions, we report on the occurrence of a photosystem I (PSI)-dependent photoreduction of NO into N₂O in the unicellular green alga *C. reinhardtii*. Through the study of mutants deficient in FLVs or CYP55 or both, we conclude that FLVs mainly contribute to N₂O production in the light, while CYP55 is mostly involved in the dark. The ecological implication of NO reduction to N₂O by microalgae, a phenomenon shown to be restricted to algae of the green lineage, is discussed.

Results

***C. reinhardtii* Reduce NO to N₂O in the Light Using the Photosynthetic Electron Transport Chain.** Measurements of NO and N₂O exchange were performed on *C. reinhardtii* cell suspensions using MIMS. To ensure sufficient amount of substrate, we injected exogenous NO into the cell suspension. Because NO can be spontaneously oxidized into nitrite in the presence of O₂, experiments were performed under anoxic conditions by adding glucose and glucose oxidase to the cell suspension as an O₂ scavenger. After NO injection, NO uptake and N₂O production were measured in the dark (Fig. 1*A* and *B*), and the NO uptake rate was found about twice higher than the N₂O production rate (Fig. 1*C*), thus indicating the existence in the dark of a stoichiometric reduction of NO into N₂O. Upon illumination, a strong increase in both NO

Significance

Nitrous oxide (N₂O), the third most important greenhouse gas in the atmosphere, is produced in great quantities by microalgae, but molecular mechanisms remain elusive. Here we show that the green microalga *Chlamydomonas reinhardtii* produces N₂O in the light by a reduction of NO driven by photosynthesis and catalyzed by flavodiiron proteins, the dark N₂O production being catalyzed by a cytochrome p450. Both mechanisms of N₂O production are present in chlorophytes, but absent from diatoms. Our study provides an unprecedented mechanistic understanding of N₂O production by microalgae, allowing a better assessment of N₂O-producing hot spots in aquatic environments.

Author contributions: A.B. and G.P. designed research; A.B., P.R., and A.G. performed research; A.B. contributed new reagents/analytic tools; A.B., Y.L.-B., and G.P. analyzed data; and A.B., Y.L.-B., and G.P. wrote the paper.

The authors declare no competing interest.

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Data deposition: Genes studied in this article can be found on <https://phytozome.jgi.doe.gov/> under the loci [Cre12.g531900](https://phytozome.jgi.doe.gov/loci/Cre12.g531900) (FLVA), [Cre16.g691800](https://phytozome.jgi.doe.gov/loci/Cre16.g691800) (FLVB), and [Cre01.g007950](https://phytozome.jgi.doe.gov/loci/Cre01.g007950) (CYP55). Sequence data from this article can be found in the GenBank data library (<https://www.ncbi.nlm.nih.gov/genbank/>) under accession numbers [XM_001699293.1](https://www.ncbi.nlm.nih.gov/genbank/acc/XM_001699293.1) (FLVA), [XM_001692864.1](https://www.ncbi.nlm.nih.gov/genbank/acc/XM_001692864.1) (FLVB), and [XP_001700272.1](https://www.ncbi.nlm.nih.gov/genbank/acc/XP_001700272.1) (CYP55).

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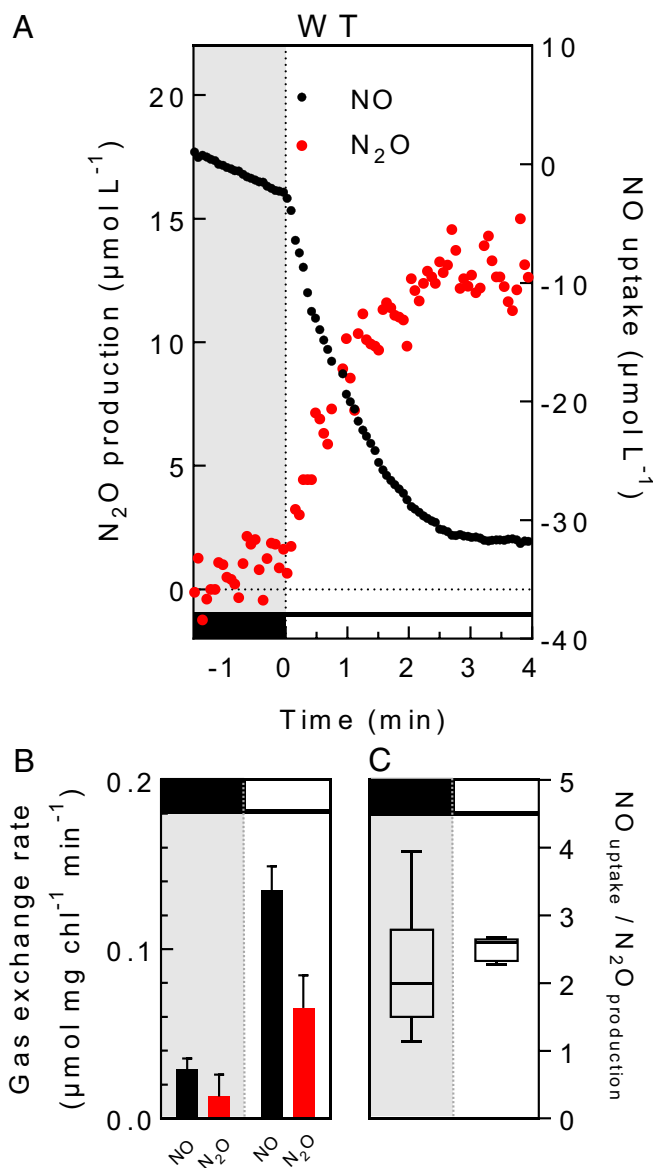


Fig. 1. Reduction of NO into N₂O in the green alga *C. reinhardtii*. After 1 min anaerobic acclimation, NO was injected in the cell suspension to a final concentration of 45 μM . After 3 min in the dark, cells were illuminated with green light ($3,000 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$). (A) Representative traces of cumulated amounts of NO uptake (black circles) and N₂O production (red circles) measured in the control *C. reinhardtii* strain during a dark to light transient. (B) Dark (Left) and light-dependent (Right) NO uptake rates (black) and N₂O production rates (red). Data shown are mean values \pm SD ($n = 4$). (C) Box plot of the ratio of NO uptake rate over N₂O production rate in the dark and over the entire light period. (mean, min, max, $n = 8$).

uptake and N₂O production was observed (Fig. 1 A and B). The NO_{uptake}/N₂O_{production} ratio increased up to a value of 2.5 (Fig. 1C). This likely indicates the occurrence of two distinct phenomena in the light: 1) a stoichiometric photoreduction of NO into N₂O, and 2) a photo-dependent NO uptake process independent of N₂O production.

In order to determine whether photosynthesis could serve as a source of electrons for NO reduction in the light, we studied the effect of two inhibitors, 3,4-dichlorophenyl-1,1-dimethylurea (DCMU), a potent photosystem II (PSII) inhibitor, and 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB), a plastoquinone analog blocking the photosynthetic electron flow

between PSII and PSI. While both inhibitors had no effect in the dark, DCMU decreased the light-dependent NO uptake and the light-dependent N₂O production rate by 65 and 55%, respectively (Fig. 2 A, C, and D). On the other hand, DBMIB decreased the light-dependent NO uptake rate by more than 90% and completely abolished the light-dependent N₂O production (Fig. 2 B–D). The above experiments performed with photosynthetic inhibitors point to the involvement of PSI in the photoreduction of NO into N₂O, the partial inhibition observed in the presence of DCMU likely resulting from the occurrence of a nonphotochemical reduction of plastoquinones by the alternative NAD(P)H dehydrogenase 2 (NDA2), as previously reported for *C. reinhardtii* (24). We conclude from these experiments that *C. reinhardtii* can reduce NO into N₂O in a light-dependent manner using electrons provided by the photosynthetic electron transport chain.

Light-Dependent N₂O Photoproduction Mostly Relies on FLVs. Depending on their origin, bacterial flavodiiron proteins are capable of catalyzing O₂ reduction, NO reduction, or both reactions (23). In *C. reinhardtii*, two FLVs (FLVA and FLVB) have recently been described as catalyzing O₂ photo-reduction using the reducing power produced at the PSI acceptor side by photosynthesis (22). In order to determine the contribution of FLVs to NO

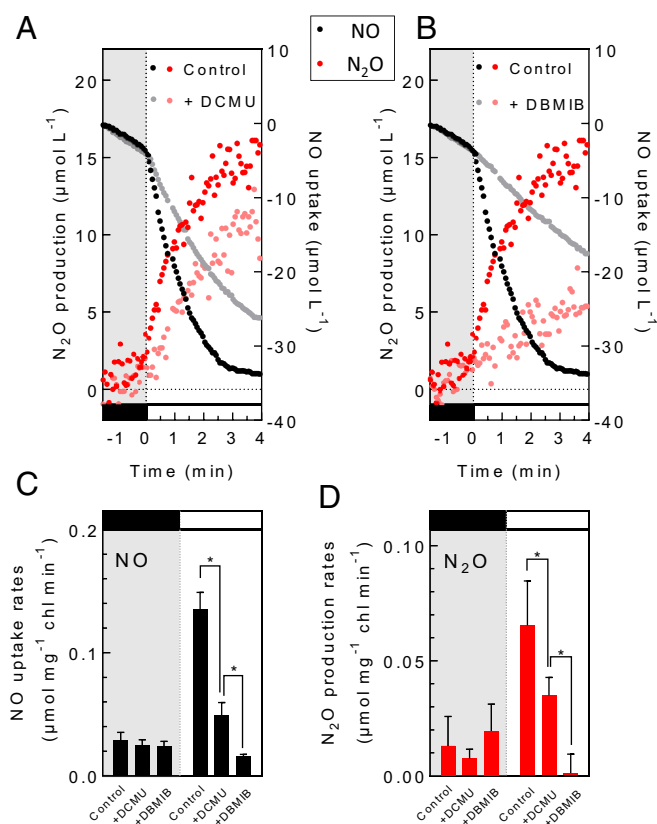


Fig. 2. The photoreduction of NO into N₂O involves the photosynthetic electron transport chain. NO and N₂O gas exchange were measured during a light transient as described in Fig. 1 in the absence or presence of photosynthesis inhibitors DCMU (10 μM) or DBMIB (2 μM). (A and B) Representative traces of cumulated NO uptake (black dots) and N₂O production (red dots) in the control strain in the absence (full color dots) or presence of DCMU or DBMIB (grayed out dots). (C) Dark (Left) and light-dependent (Right) NO uptake rates measured in the absence or presence of inhibitors. (D) Dark (Left) and light-dependent (Right) N₂O production rates measured in the absence or presence of inhibitors. Data shown are mean values \pm SD ($n = 4$). Asterisks mark significant differences ($P < 0.05$) based on multiple *t* tests.

photoreduction, we analyzed NO and N₂O exchange during dark to light transients in three previously characterized *flvB* mutants (22). These mutants are impaired in the accumulation of both FLVB and FLVA subunits (22, 25, 26). In the dark, no significant difference in neither NO reduction nor in N₂O production were found between the three *flvB* mutants as compared to the control (Fig. 3 A, C, E, and F and *SI Appendix, Fig. S1 A and B*). In contrast, the N₂O production and NO uptake induced by light were, respectively, decreased by 70 and 50% in *flvB-21* as compared to the control strain (Fig. 3 A, C, E, and F). Similar effects were observed in the two other independent *flvB* mutants (*SI Appendix, Fig. S1 A and B*). We conclude from this experiment that FLVs are involved in the light-dependent reduction of NO into N₂O, using electrons produced by photosynthesis.

Dark N₂O Production Relies on CYP55. A homolog of the nitric oxide reductase from *Fusarium oxysporum* (CYP55) encoded by the *C. reinhardtii* genome (Cre01.g007950) was recently proposed to be involved in NO reduction (13). In order to investigate the contribution of the CYP55 homolog to N₂O production, we obtained three *C. reinhardtii* insertion mutants from the CLiP library (<https://www.chlamylibrary.org> (27)). Two of these putative *cyp55* mutants have been predicted to hold an insertion of the paromomycin resistance cassette in introns, while the third one has a predicted insertion in an exon (*cyp55-95*) (*SI Appendix, Table S1 and Fig. S2A*). Positions of insertions were confirmed by PCR on genomic DNA (*SI Appendix, Fig. S2 B and C*). Both

NO uptake and N₂O production in the dark were completely abolished in all three *cyp55* mutants (Fig. 3 A, B, E, and F and *SI Appendix, Fig. S1*). However, rates of NO reduction and N₂O production induced by light were not significantly affected in all three *cyp55* mutants (Fig. 3 A, B, E, and F and *SI Appendix, Fig. S1*). We conclude from this experiment that CYP55 is responsible for the entire reduction of NO to N₂O in the dark.

Furthermore, a double mutant with mutations in both *CYP55* and *FLVB* was obtained by crossing *cyp55-95* and *flvB-21* strains, and three independent progenies (*flvB cyp55-1*, -2, and -3) were isolated (*SI Appendix, Fig. S3*). The N₂O production was nearly abolished in these double mutant lines both in the dark and in the light (Fig. 3 D and F and *SI Appendix, Fig. S4B*). The quantity of N₂O produced during the first minute of illumination was significantly lower in the double mutants when compared to the single *flvB-21* mutant (*SI Appendix, Fig. S5*), so was the NO uptake rate (Fig. 3E and *SI Appendix, Fig. S4A*). Thus, in the absence of FLVs, CYP55 contributes to the light-dependent NO reduction to N₂O production, which is consistent with the predicted chloroplast targeting of CYP55 (*SI Appendix, Fig. S6*).

N₂O Production by FLVs and CYP55 Occurs under Aerobic Conditions in the Presence of an Internal Source of NO. In previous experiments, NO reduction has been assayed under forced anaerobic conditions following the addition of exogenous NO. However, in natural environments, algae mostly experience aerobic conditions, and NO is produced within cells during the reduction of nitrates (NO₃⁻) or nitrites (NO₂⁻) (2). The following experiments were then carried

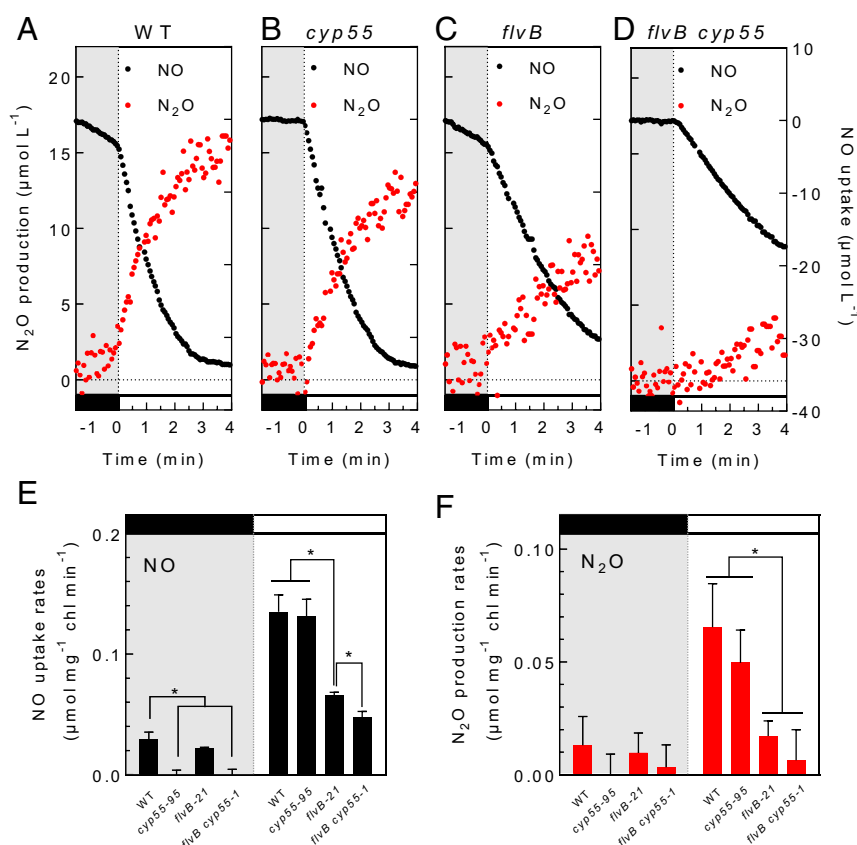


Fig. 3. The light-dependent N₂O production involves FLVs while the dark production involves CYP55. NO and N₂O gas exchange were measured during a light transient as described in Fig. 1 in the control strain (A), the *cyp55-95* mutant deficient in CYP55 (B), in the *flvB-21* mutant deficient in FLVB (C), and in a double *flvB cyp55-1* mutant (D). (A–D) Representative traces of cumulated NO production (black) and N₂O production (red). (E) Dark (Left) and light-dependent (Right) NO uptake rates. (F) Dark (Left) and light-dependent (Right) N₂O production rates. Data shown are mean values ± SD (n = 4). Asterisks mark significant differences (P < 0.05) based on multiple t tests.

out in order to determine whether reduction of NO into N₂O by FLVs and CYP55 can also be evidenced in conditions more representative of natural environments. We first verified that both dark and light-induced NO reduction phenomena were still present in strains grown with NO₃[−] as the nitrogen source (*SI Appendix*, Fig. S7). Since FLVs are able to reduce both NO and O₂ (17), we then examined the effect of O₂ on N₂O photoproduction in the *cyp55-95* mutant (*SI Appendix*, Fig. S8). When exogenous NO was supplied at a concentration of 45 μM, the N₂O production rate was only slightly inhibited by O₂ concentrations up to 100 μM and reached about 50% of its initial value at an estimated O₂ concentration close to atmospheric O₂ concentration (250 μM O₂). This experiment shows that FLV-mediated NO photoreduction occurs at significant rates in the presence of O₂ and further indicates that *C. reinhardtii* FLVs have a relatively higher affinity for NO than for O₂.

In order to determine whether N₂O production can be evidenced in the absence of exogenous NO supply, algae were supplied with nitrite as a nitrogen source, nitrite reduction being documented as an important source of intracellular NO (28). Note that we did not use nitrate here since the *C. reinhardtii* mutant strains used in this study all lack the nitrate reductase (27). In the presence of nitrite, both NO and N₂O were produced in the dark by wild-type (WT) cells. Upon illumination, NO was quickly consumed and the N₂O production rate increased (Fig. 4). Note that N₂O production rates measured in these conditions were lower than in previous experiments (Figs. 1–3), partly due to the presence of a 10 times lower initial NO concentration (4–6 μM as compared to 45 μM) (*SI Appendix*, Fig. S9), and partly due to the competition between NO and O₂ for FLV-mediated photoreduction (*SI Appendix*, Fig. S8). Both dark and light-induced N₂O production were completely abolished in the *flvB cyp55-1* double mutant (Fig. 4), and the dark NO production rate was higher than in the control strain (Fig. 4 and *SI Appendix*, Fig. S9). Similar experiments were performed on single mutants (*SI Appendix*, Fig. S10), leading to conclude that both CYP55 and FLVs are responsible for N₂O production in aerobic conditions when NO is produced endogenously from the reduction of nitrites. Importantly, the increase in NO production rates observed in the absence of CYP55 in the dark, or the lower NO decrease observed in the absence of FLVs in the light (Fig. 4 and *SI Appendix*, Figs. S9 and S10) indicate that both CYP55 and FLVs contribute to the intracellular NO homeostasis, respectively, in the dark and in the light.

N₂O Production Is Restricted to Chlorophytes and Correlates with the Presence of FLV and CYP55. We then explored the ability of microalgae originating from different phyla to reduce NO to N₂O either in the dark or in the light. We focused on species with sequenced genomes or transcriptomes and listed the presence of *FLV* and *CYP55* homologous genes in the different species (*SI Appendix*, Table S3). The ability to produce N₂O in the dark was only found in algae of the green lineage (chlorophytes) (*SI Appendix*, Fig. S9) and associated with the presence of a *CYP55* gene homolog (Fig. 5). A light-dependent N₂O production was also only found in chlorophytes (*SI Appendix*, Fig. S11) and associated with the presence of *FLV* genes (Fig. 5). Note that diatoms and red algae showed a light-dependent NO uptake but without significant N₂O production (*SI Appendix*, Fig. S11 E–I). Similar light-dependent NO uptake was also evidenced in the *C. reinhardtii flvB cyp55* double mutants (Figs. 3 and 4 and *SI Appendix*, Fig. S4), thereby highlighting the existence in all analyzed algal species of a mechanism of photo-dependent NO uptake, which likely reflects oxidation of NO by the PSII-produced O₂. We conclude from these experiments that N₂O production in microalgae mostly relies on CYP55 in the dark and on FLVs in the light.

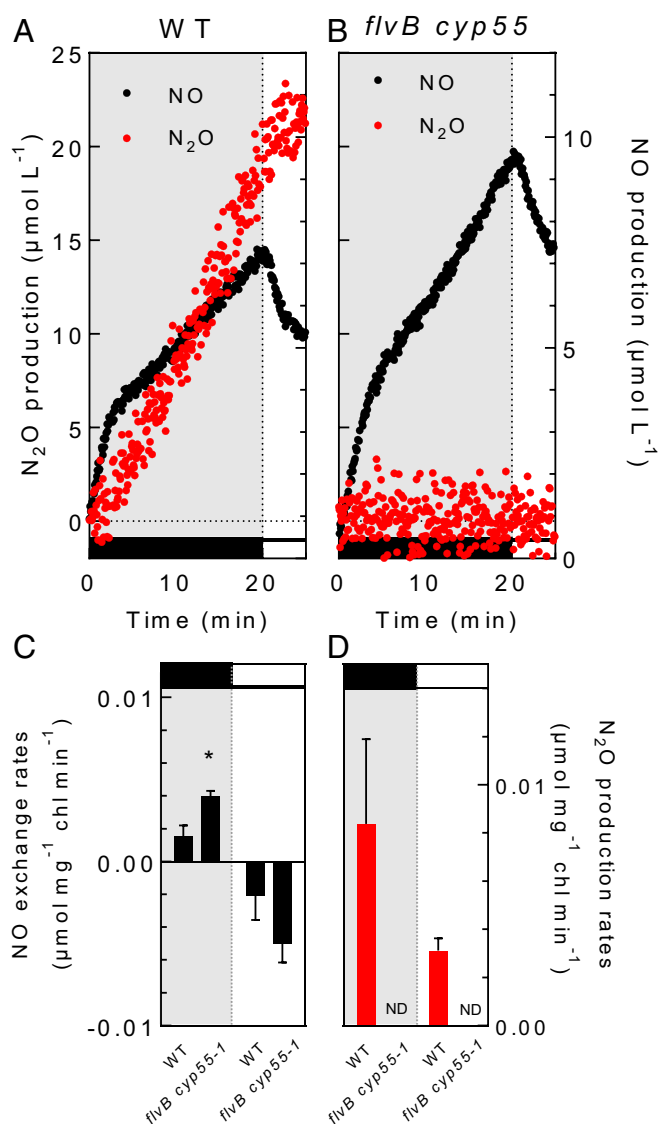


Fig. 4. N₂O production by FLVs and CYP55 in the absence of external NO supply. NO and N₂O gas exchange were measured during a dark to light transient after injection of 10 mM NO₂[−] to a final concentration at $t = 0$. Control strain (A), *flvB cyp55-1* double mutant (B). (A and B) Representative traces of cumulated NO production (black) and N₂O production (red). (C and D) Dark (Left) and light-dependent (Right) NO and N₂O exchange rates, respectively. Data shown are mean values \pm SD ($n = 3$). Asterisks mark significant differences ($P < 0.05$) based on multiple t tests. The O₂ concentration before illumination ranged from 35 to 55 μM. Similar data obtained for *cyp55-95* and *flvB-21* simple mutants are shown in *SI Appendix*, Fig. S10. ND, not detected.

Discussion

Although the ability of green algae to produce N₂O had been documented for more than 30 y (29), molecular mechanisms remained enigmatic. In this work, we show that the green microalga *C. reinhardtii* can reduce NO to N₂O in the dark as well as in the light but employing different mechanisms. The light-dependent reduction of NO is catalyzed by FLVs and uses electrons produced by the photosynthetic chain while the dark reaction is mediated by CYP55. Both reactions occur when NO is produced endogenously and therefore participate in the intracellular NO homeostasis.

In microalgae and land plants, the photosynthetic electron flow is principally used to reduce CO₂ via the Calvin-Benson cycle. Several alternative electron fluxes also occur, which usually play critical roles during acclimation of photosynthesis to environmental

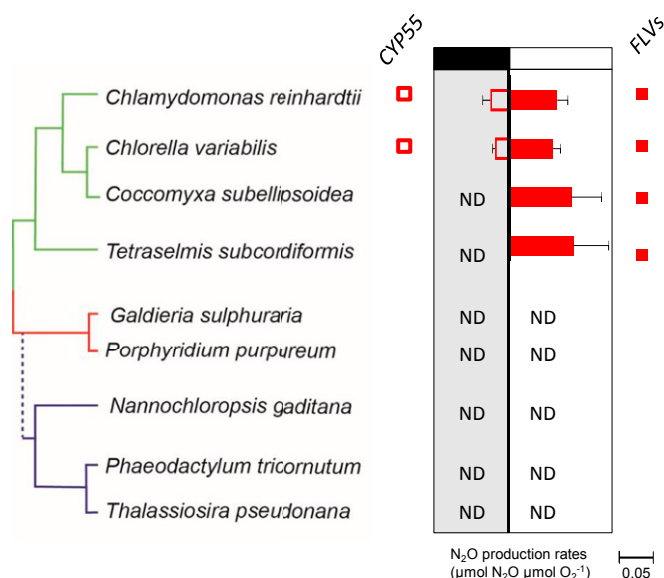


Fig. 5. The light-dependent N₂O production is associated with the presence of FLVs while N₂O production in the dark is associated with the presence of CYP55 in algal genomes. Presence of CYP55 and FLV homologous genes in different algal species are respectively shown by open or filled red squares. NO and N₂O gas exchange were measured in the different species during a dark-light transient as described in Fig. 1. Maximal gross O₂ production rate by PSII was measured on the same samples using labeled ¹⁸O₂. When detected, N₂O production rates were normalized to the corresponding maximum gross O₂ production rates. Relative rates of dark (Left) and light-dependent (Right) N₂O production are shown. Data shown are mean values ± SD (n = 3). ND, not detected.

changes (30–32). Among known electron acceptors of alternative electron fluxes, one finds: 1) molecular O₂ that can be reduced into water or reactive oxygen species by different mechanisms (33), 2) protons that can be reduced into dihydrogen by hydrogenases (34), and 3) nitrites reduced into ammonium by nitrite reductases (35). We provide evidence here that NO is to be considered as an electron acceptor of algal photosynthesis downstream PSI. Since FLVs of photosynthetic eukaryotes are closely related (36), we anticipate that NO photoreduction also occurs in mosses and other photosynthetic eukaryotes harboring FLV genes. The maximal proportion of the photosynthetic electron flow used for NO reduction is estimated around 5% considering that at PSII four electrons are produced per molecule of O₂ released, and that two electrons are used per molecule of N₂O produced. Due to this relatively low rate, it seems unlikely that NO photoreduction significantly functions as a valve for electrons during the functioning of photosynthesis, as it has been shown for O₂ photoreduction by FLVs under aerobic conditions (22) or H₂ photoproduction under anaerobic conditions (37). Nevertheless, NO photoreduction could play a regulatory role during anaerobic photosynthesis, as shown for other minor pathways such as chlororespiration under aerobic conditions (31, 38). In photosynthetic organisms, FLVs have so far been solely involved in oxygen reduction; this electron valve being critical for growth under fluctuating light conditions (22, 26, 39, 40). We show here that FLVs, by reducing O₂ or NO, play a dual function in chlorophytes. Although the ability to reduce NO might be regarded as an unnecessary reaction representing a relic of the evolution of their bacterial ancestors, the higher affinity of *C. reinhardtii* FLVs for NO than for O₂ (SI Appendix, Fig. S8) suggests the existence of a selective pressure that maintained and even favored the NO reduction reaction. The physiological significance of NO photoreduction by FLVs, which may be related to their

involvement in NO homeostasis, will need further investigation to be elucidated.

In microalgae, N₂O production occurs mostly during nitrogen assimilation, when nitrates or nitrites are supplied as nitrogen sources (12). During this process, nitrite is reduced into ammonium by the nitrite reductase (35), but can alternatively be reduced to NO by either a NO-forming nitrite reductase (41) or by respiratory oxidases (42). N₂O was observed in conditions where NO is produced and CYP55 was suggested to be involved in its reduction (13). Our work establishes that the CYP55 is indeed involved in this process, but essentially in the dark. Production of N₂O by microalgal cultures was also reported to greatly vary depending on culture conditions like illumination, nitrogen source, or oxygen availability (10, 12, 29), which remained mostly unexplained (13). Our results clearly demonstrate the existence of two distinct pathways of NO reduction characterized by contrasted properties of light dependence and O₂ sensitivity. The variability in N₂O production may therefore result from variation in the relative importance of both CYP55 and FLVs reductive pathways depending on experimental conditions.

NO is a known signal molecule involved in various regulatory mechanisms in all living organisms. In microalgae, NO is involved in the regulation of the nitrate and nitrite assimilation pathways (43, 44), in the down-regulation of photosynthesis upon nitrogen and sulfur starvation (45, 46), in hypoxic growth (47), or during acclimation to phosphate deficiency (48). NO homeostasis results from an equilibrium between NO production by different enzymatic systems, which have been well documented in plants (49), and its active degradation mediated by different mechanisms including truncated hemoglobin that catalyze NO oxidation to nitrate in aerobic conditions (50, 51). Our results suggest that FLVs and CYP55, by removing NO through reductive pathways, are key enzymes in the control of NO homeostasis both in anaerobic and aerobic conditions.

Microalgae are often considered promising organisms for the production of next-generation biofuels provided that their large-scale cultivation has positive effects on the environment. However, it has recently been estimated that N₂O produced during large-scale algal cultivation could compromise the expected environmental benefits of algal biofuels (14). In this context, knowledge of the molecular mechanisms involved and the selection of microalgal species with limited N₂O production capacity are essential to limit the global warming potential of algal biofuels.

To date, the contribution of microalgae to the N₂O atmospheric budget and global warming is not taken into account (10) due to our lack of knowledge about algal species concerned and the conditions of N₂O production. We have shown that the capacity to reduce NO into N₂O greatly depends on algal species, and is essentially restricted to chlorophytes, the second most represented photosynthetic organisms in the ocean (52, 53). Chlorophytes are particularly abundant in coastal waters (52), where anthropic releases contain high concentrations of inorganic nitrogen (54, 55) and favor hypoxia (56), thus promoting N₂O production (57). Coastal waters are frequently the scene of N₂O-producing hot spots resulting from the accumulation of phytoplanktonic biomass (58) likely due to the NO-reductive activity of chlorophytes. The incoming worldwide N₂O observation network (59) together with follow up of phytoplanktonic communities will be decisive to better assess the microalgal contribution, particularly chlorophytes, to the global N₂O budget.

Materials and Methods

C. reinhardtii strains were grown mixotrophically under dim light (5–10 μmol photons m⁻² s⁻¹) in Tris-acetate-phosphate medium (TAP) when not specified otherwise. The *C. reinhardtii* wild-type strain CC-4533 and *flvB* mutants (*flvB-21*, *flvB-208*, and *flvB-308*) were previously described (22). The *cyp55* mutants were obtained from the CLiP library (27). Other algal species and their

respective culture media are listed in [SI Appendix, Table S3](#). Gas exchange rates were measured using a membrane inlet mass spectrometer.

Data and Materials Availability. All data needed to evaluate the conclusions in the paper are present in the paper and/or the [SI Appendix](#).

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