



Cryptic oxygen cycling in anoxic marine zones

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Oxygen availability drives changes in microbial diversity and biogeochemical cycling between the aerobic surface layer and the anaerobic core in nitrite-rich anoxic marine zones (AMZs), which constitute huge oxygen-depleted regions in the tropical oceans. The current paradigm is that primary production and nitrification within the oxic surface layer fuel anaerobic processes in the anoxic core of AMZs, where 30–50% of global marine nitrogen loss takes place. Here we demonstrate that oxygenic photosynthesis in the secondary chlorophyll maximum (SCM) releases significant amounts of O₂ to the otherwise anoxic environment. The SCM, commonly found within AMZs, was dominated by the picocyanobacteria *Prochlorococcus* spp. Free O₂ levels in this layer were, however, undetectable by conventional techniques, reflecting a tight coupling between O₂ production and consumption by aerobic processes under apparent anoxic conditions. Transcriptomic analysis of the microbial community in the seemingly anoxic SCM revealed the enhanced expression of genes for aerobic processes, such as nitrite oxidation. The rates of gross O₂ production and carbon fixation in the SCM were found to be similar to those reported for nitrite oxidation, as well as for anaerobic dissimilatory nitrate reduction and sulfate reduction, suggesting a significant effect of local oxygenic photosynthesis on Pacific AMZ biogeochemical cycling.

Prochlorococcus | oxygen minimum zone | secondary chlorophyll maximum | metatranscriptomics | aerobic metabolism

In coastal zones of the eastern tropical Pacific Ocean, the upward transportation of nutrient-rich waters results in relatively high primary productivity at surface depths. Sinking of organic matter produced by surface production coupled with sluggish circulation leads to the formation of oxygen-deficient water masses at intermediate depths below the mixed layer. Due to strong stratification, these oxygen minimum zones (OMZs) extend far offshore over vast swaths of the eastern Pacific. In these regions, oxygen availability plays a major role in structuring organism distributions and biogeochemical processes in the pelagic ocean (1).

Recently developed sensor techniques (2) show that in much of the OMZ water column, from about 30–100 m to about 800 m, O₂ concentrations fall below sensor-specific detection limits of down to 3 nmol·L⁻¹ (3·10⁻⁹ moles per liter) (3, 4). OMZs in the eastern tropical North and South Pacific (ETNP and ETSP, respectively) and in the Arabian Sea are subject to such intense O₂ depletion and therefore have been redefined as anoxic marine zones (AMZs) (5). In other oceanic OMZs, including in the Bay of Bengal and northeast Pacific, oxygen concentrations may decrease to a few micromolar, but total O₂ depletion occurs only occasionally (6). AMZs are often distinguished from more oxygen-replete OMZs by the accumulation of nitrite, which is typically most pronounced when O₂ falls below the nanomolar detection limit (5–8). Nitrite is a key substrate in microbial N₂ and N₂O production by either denitrification or anaerobic ammonium oxidation (anammox), which together in AMZs mediate 30–50% (9) of the marine recycling of inorganic nitrogen compounds (nitrate, nitrite, and ammonium) to atmospheric N₂.

Nitrite is also produced and consumed in the aerobic nitrification pathway involving the two-step process of aerobic ammonia and nitrite oxidation (10, 11). Despite the absence of measureable O₂ in the core of eastern Pacific AMZs, biomolecular evidence (DNA, RNA, and proteins) indicates the presence of aerobic microbial processes. The expression of genes encoding for nitrification and other O₂-dependent microbial metabolisms, potentially including heterotrophic respiration, have been found well below the oxycline (12, 13), raising the question of how aerobic processes could persist under apparent anoxia.

In the three oceanic AMZs of the Arabian Sea, ETNP, and ETSP, dense populations of phototrophs have been observed at the base of the photic zone but below the oxycline that separates oxic from anoxic waters (14–16). This deep secondary chlorophyll maximum (SCM) is mainly composed of novel, yet uncultivated, lineages of the cyanobacterium *Prochlorococcus* (14), with chlorophyll concentrations that can equal that of the primary chlorophyll peak near the surface (16). The presence of this large population of putative oxygenic phototrophs has suggested a mechanism by which aerobic metabolism can be maintained in a zone where in situ measurements indicate anoxic conditions (5). Although an active photosynthetic community produces and releases oxygen to the environment, coupled O₂ consumption by an aerobic microbial community may keep seawater O₂ concentration at very low and possibly subnanomolar levels, thereby resulting in a cryptic O₂ cycle. The existence of such a cryptic

Significance

Anoxic marine zones (AMZs) create expansive habitats for microbes whose anaerobic metabolisms help drive global nutrient cycles, for example, by removing nitrogen from the oceans by producing N₂ gas. AMZ cycles may also be shaped by oxygen intrusion from outside the AMZ, creating opportunities for aerobic microbial metabolisms. Here we show that aerobic processes in AMZs are linked to oxygen production within the anoxic zone. Oxygen is produced during daytime in a layer of photosynthetic cyanobacteria near the top of the AMZ and then rapidly consumed by aerobic processes without accumulating. Oxygen turnover and carbon fixation rates are comparable to those of microbial N₂ production, suggesting an important role for internal oxygen cycling in AMZ transformations of matter and energy.

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Data deposition: The sequences have been deposited in the National Center for Biotechnology Information (NCBI, www.ncbi.nlm.nih.gov). For a list of accession numbers, see Table S5.

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oxygen cycle has been suggested by biomolecular evidence (12) but has not yet been demonstrated.

In this study we used a combination of high-resolution oxygen profiling, metabolic rate measurements, and community mRNA sequencing to explore the potential for oxygen cycling in the SCMs of the ETNP off Mexico and the ETSP off Peru. Our results show that the photosynthetic community of the SCM produces significant amounts of O_2 , sufficient to maintain an aerobic community in an otherwise anoxic environment. Rates of O_2 production and carbon fixation in the SCM in both ETNP and ETSP AMZs are comparable to previously measured rates of aerobic processes like nitrite and ammonium oxidation (8, 17), as well as anaerobic AMZ processes like denitrification, anammox, and sulfate reduction (7, 8). Although the measured metabolic rates exhibit large spatial and temporal variability, our data collectively suggest a significant effect of local photosynthesis on the biogeochemical cycling in Pacific Ocean AMZs.

Results and Discussion

Sampling in both the ETNP and ETSP revealed a typical AMZ O_2 distribution in the upper 200 m of the water column. Oxygen concentrations in the 0–35 m surface layer in the ETNP were stable at $\sim 200 \mu\text{mol}\cdot\text{kg}^{-1}$, before declining along a clearly defined oxycline from 35–45 to 60–80 m, and then falling below the detection limit of the switchable trace amount oxygen (STOX) sensors (few nanometers) at 80–100 m (Fig. 1B). In the ETSP AMZ off Peru, O_2 concentrations and the depth of the oxycline were more variable and clearly influenced by proximity to the shore, with anoxic depths beginning at ~ 30 m at the coastal station but at ~ 70 m for the more oceanic station (Fig. 1H). In both the ETNP and ETSP, the chlorophyll concentration below

the primary maximum decreased in parallel with O_2 concentration, reaching a minimum before complete O_2 depletion and then increasing again to form an SCM in which 90% of the phototrophs were *Prochlorococcus* (Fig. 1 and Table S1). Although the upper region of the SCM was consistently located near the oxic–anoxic interface, maximum in vivo fluorescence and *Prochlorococcus* abundance were usually localized within the anoxic zone a few meters below. Low O_2 concentrations ($<500 \text{ nmol}\cdot\text{L}^{-1}$) were occasionally found inside the SCM (Table S2), suggesting intrusion of overlying oxygenated waters or in situ O_2 production and accumulation.

Oxygenic Photosynthesis and Carbon Fixation in the SCM. Shipboard experiments using water from the SCM incubated under trace O_2 conditions revealed that O_2 concentration with time differed substantially between dark- and light-incubated samples (Fig. 2A and D). Net community production (NCP), corresponding to the slope of the O_2 concentration curves and hence the balance between O_2 production and consumption, gradually increased to more positive values with increasing irradiance. NCP was also variable between stations, reflecting the spatial and temporal variability of the metabolic activity in terms of photosynthesis and respiration rates (Fig. 2B and E). At several stations, net consumption of O_2 occurred at all applied irradiances, although a clear decrease in consumption rate was always measured with increasing light intensities. At other stations, a net increase in O_2 was measured when the samples were exposed to an irradiance of only $10 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The observed maximum irradiance in situ was, however, only in the range of $2\text{--}5 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at most stations (see examples in Fig. S1); at such low light levels, net O_2 consumption was always observed.

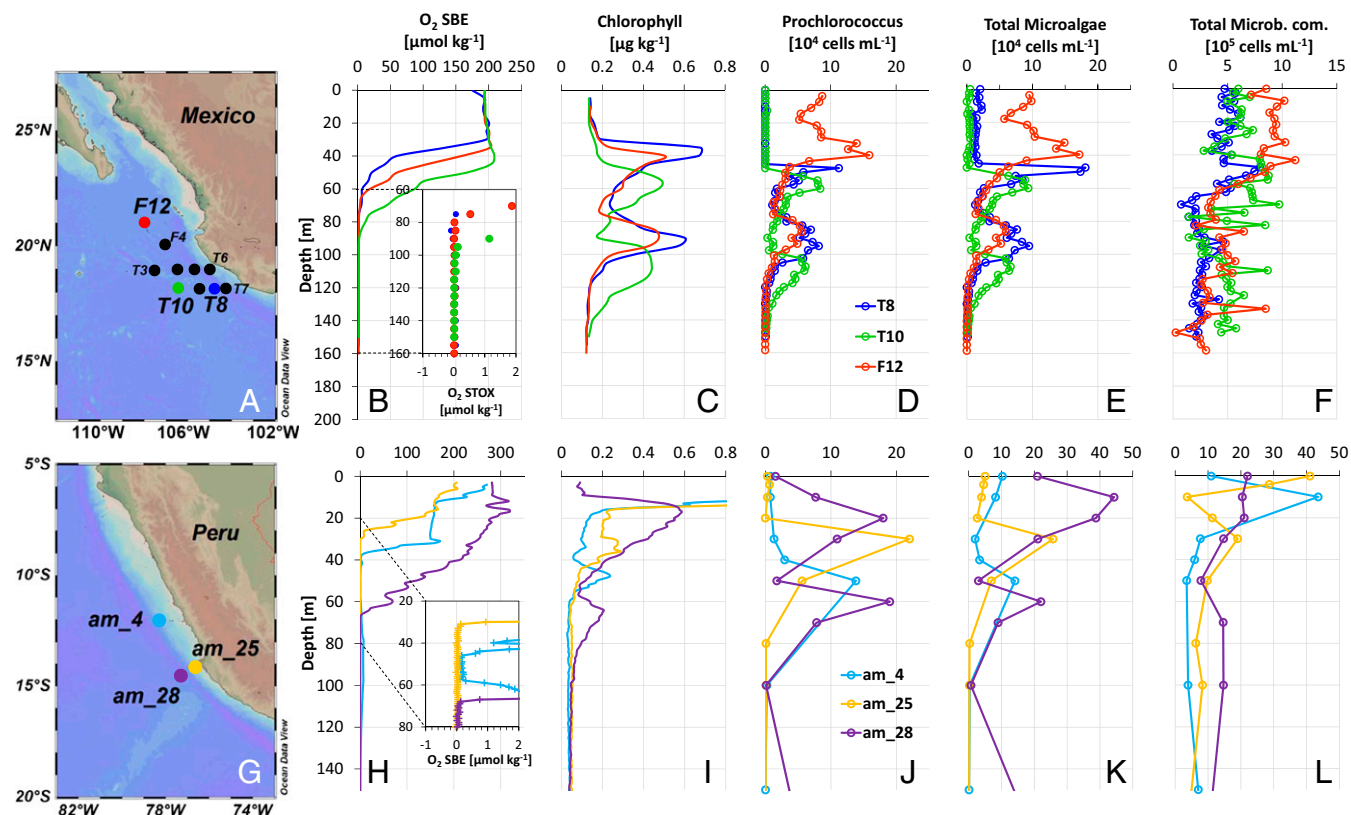


Fig. 1. Maps with sampled stations and main characteristics of the upper part of the (A–F) ETNP AMZ and (G–L) ETSP AMZ. Stations off Mexico (A) and Peru (G) where the SCM was found and sampled. (B and H) Dissolved oxygen profiles, based on SBE43 and STOX sensors (zooming in at low STOX O_2 values in B or corrected SBE O_2 in H). (C and I) Profiles of chlorophyll concentration inferred from in vivo fluorescence. (D and J) *Prochlorococcus* abundance. (E and K) Total microalgae (*Prochlorococcus*, *Synechococcus*, and picoeukaryotes) and (F and L) total microbial community (Total Microb. com.) abundance measured by flow cytometry.

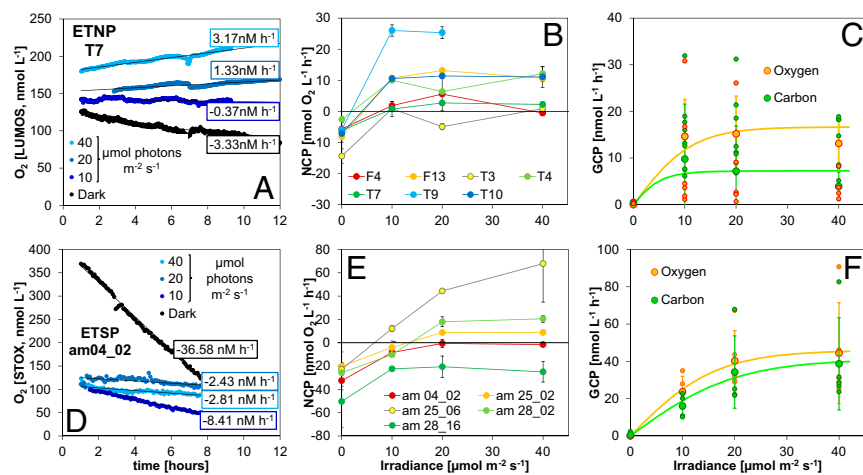


Fig. 2. Oxygen production and carbon fixation during incubations of samples from the SCM off (A–C) Mexico and (D–F) Peru. (A and D) Evolution of O₂ concentration during incubation of SCM samples exposed to a range of scalar irradiances (0–40 μmol photons·m⁻²·s⁻¹). (B and E) Net community production (NCP) rates versus scalar irradiance. (C and F) Gross community production (GCP): O₂-GCP was measured as the net O₂ production, and C-GCP was measured by the incorporation of ¹³C (at ETNP) or ¹⁴C (ETSP). Data were fitted to a photosynthesis–irradiance model to calculate maximum rates (GCP_{max}) and the initial slope of the curve (α), an index of the photosynthetic efficiency at low light (values in the main text). Error bars represent the SE.

Tracking O₂ consumption during our experiments allowed for estimates of aerobic respiration rates. O₂ consumption curves were linear down to about 50 nmol·L⁻¹ during dark incubations (Fig. 2 A and D). Aerobic respiration by prokaryotes is generally driven by two classes of terminal oxidases: low-affinity terminal oxidases (LATO) with a half saturation constant (K_m) of about 200 nmol O₂·L⁻¹ and high-affinity terminal oxidases (HATO) with K_m values of 3–8 nmol O₂·L⁻¹ (18). Marine bacteria possessing HATO can decrease apparent K_m values of aerobic respiration down to less than 10 nmol O₂·L⁻¹ (19), and a linear O₂ decrease may thus be expected down to about 50 nmol O₂·L⁻¹. Therefore, O₂ consumption rates (referred to as respiration for simplicity) obtained at concentrations >50 nmol·L⁻¹ represent potential respiration rates (R^*) because they were measured above the threshold of O₂ limitation. The estimated R^* rates were significantly higher in the ETSP compared with the ETNP (Fig. 2 and Table S2), consistent with a higher microbial and particle abundance measured in the ETSP (Fig. 1 and Tables S1 and S2).

Experiments under the unique, almost anoxic conditions, of AMZs have not been performed in previous measurements of photosynthetic activity in the SCM (20). We conducted our experiments at O₂ levels below those sporadically detected by in situ measurements (up to 500 nmol·L⁻¹) but far above the K_m values for HATO. In this range, we can assume that gross community production of O₂ (GCP-O₂) can be calculated as the sum of NCP and R^* . We also validated these production calculations by simultaneously measuring the incorporation of inorganic carbon (using ¹³C or ¹⁴C) into biomass [gross community carbon production (GCP-C)], as has been done previously to quantify *Prochlorococcus* carbon fixation (20, 21). Both GCP-O₂ and GCP-C followed a classical photosynthesis–irradiance curve (Fig. 2 C and F), with maximum (GCP_{max}) values above saturating light intensities (E_k) of 10.5 ± 2.0 and 21.4 ± 9.3 μmol photons·m⁻²·s⁻¹ (0.5 and 1% of the incident light) for the ETNP and ETSP, respectively. The low E_k values reflect adaptation to the dim light environment, being similar to values found for the SCM community in the Arabian Sea (20) or in *Prochlorococcus* cultures (21). Above E_k , mean GCP_{max}-O₂ values in the ETNP and ETSP were 16.6 ± 9.1 and 52.5 ± 30.4 nmol O₂·L⁻¹·h⁻¹, respectively, and were generally consistent with maximum GCP-C rates (GCP_{max}-C: 8.1 ± 11.2 and 44.4 ± 30.3 nmol C·L⁻¹·h⁻¹ in the ETNP and ETSP, respectively) (mean values of all stations ± SD in Table S3 and model in Fig. 2 C and F). The parameters describing the photosynthesis characteristics of the SCM community (maximum gross production rates, photosynthetic efficiency, and E_k) were similar to the values previously found for the SCM community of the Arabian Sea and the characterization of several *Prochlorococcus* isolates from the Pacific Ocean (20, 21). The values found for the ETNP were similar to those found

for the SCM of the Arabian Sea (20), whereas the ETSP SCM values were more similar to those from the laboratory cultures.

Although it is not yet possible to directly quantify in situ O₂ transformations in the AMZ, in situ GCP rates can be estimated based on water column chlorophyll concentrations and light conditions (Fig. S1). The light intensity at the SCM was variable and almost always substantially below 10 μmol photons·m⁻²·s⁻¹. Under such conditions, O₂ production rates are lower than potential respiration rates (R^*), and the O₂ produced is immediately consumed by the microbial community, resulting in a cryptic O₂ cycle in the seemingly anoxic environment of the SCM (Fig. 1). However, at some stations the irradiance in the SCM was similar or close to the E_k . The occasional detection of low O₂ concentrations in the SCM (4, 8) (Table S2) may thus be explained by photosynthetic activity in the SCM increasing O₂ concentrations to measurable levels. Such daily changes are difficult to measure by discrete sampling, but recurrent measurements in the same water mass might reveal hourly and daily changes in the SCM.

Oxygen Production Coupling with Aerobic Microbial Processes. Even if undetectable, O₂ production in the SCM may support important (micro)aerobic metabolisms. To explore this prediction, we looked for signatures of such aerobic metabolism in available metatranscriptomes along the AMZ depth gradient in the ETNP during two cruises in 2014 and 2013, focusing on station T6 where the SCM was well developed and for which the metatranscriptome dataset was most comprehensive. Transcripts encoding terminal oxidases, including both LATO and HATO (Table S4), were detected at all depths (Fig. 3 and Fig. S2), including deep within the AMZ, where the transcript pool was dominated by sequences affiliated with diverse Gammaproteobacteria and Alphaproteobacteria (Fig. S3). The presence of oxidase transcripts within anoxic marine waters has been reported previously (13) and may reflect constitutive expression by groups at high abundance in the AMZ core, potentially to capitalize quickly on O₂ if it becomes available (22). The relative abundance of both LATO and HATO transcripts exhibits a local peak within the SCM compared with depths immediately above (base of oxycline) and below the SCM (Fig. 3 and Fig. S2). Similar trends were observed at stations T4 and T10, although limited sampling affected our ability to fully resolve oxidase distributions immediately above the SCM at these sites (Fig. S2). Together, these data provide evidence of a local peak in O₂ scavenging within the SCM.

Oxygen produced in the SCM may also be consumed through key steps of the OMZ nitrogen cycle. Comparatively high rates of autotrophic nitrification (ammonia and nitrite oxidation) are known to occur close to the oxic–anoxic boundary of AMZs (10). Here transcripts affiliated with ammonia oxidizing bacteria

and therefore, the production values should be taken as maximum values. Productivity was also highly variable among sites (0.43–1.70/0.15–0.95, and 0.03–0.91/0.02–0.87 mmol O₂/C·m⁻²·d⁻¹ for ETNP and ETSP, respectively), reflecting the heterogeneous spatial distribution of the SCM (Fig. 1 and Tables S1 and S2).

Although primary production in surface waters largely exceeds these values (23), the vast majority of surface production is remineralized before reaching the AMZ core. Indeed, the range of particulate organic carbon supply to the AMZ is 0.83–7.81 mmol C·m⁻²·d⁻¹ (11, 24) in the ETNP or 1.52–14.70 mmol C·m⁻²·d⁻¹ in the ETSP (25). This wide range highlights the variability in export rates in these regions. Nonetheless, comparing these estimations with our data, the carbon production in the SCM could provide 5–47% and 2–20% of the organic matter supplied to the anoxic waters of the ETNP and ETSP, respectively, where part of it is then mineralized by dissimilatory nitrate reduction to nitrite and denitrification (8, 11, 23, 26). Nitrate respiration to nitrite appears as the dominant mineralization step in the ETNP (8), and mineralization rates of about 1 mmol C·m⁻²·d⁻¹ can be calculated from published data (7, 23). These rates are close to the C fixation rate in the SCM, highlighting the relevance of the SCM in OMZ metabolism.

Global warming is expected to result in shoaling of the OMZ oxycline and overall expansion of OMZ volumes (27). Mesoscale physical processes such as local upwelling and anticyclonic eddies that shoal the oxic–anoxic boundary have been shown to enhance the development of SCMs (15, 16). Oxycline shoaling increases the light intensities in the anoxic cores of the AMZs, thereby potentially stimulating the photosynthetic community. The effects of these changes on microbial communities and microbial biogeochemical cycling in AMZs are difficult to predict, although significant changes in carbon, nitrogen, and sulfur cycling are expected (27). Our data show a significant carbon supply to the anoxic core of the Pacific AMZs by SCM photosynthetic activity, and it is likely that the situation is similar in the Arabian Sea. Although we did not measure nitrogen transformation processes, the nitrifying community was also enriched at the SCM, potentially reflecting elevated metabolic rates. A shoaling of the AMZ coupled with increases in irradiance and SCM photosynthetic activity would increase the carbon and daytime oxygen supply to the upper part of the AMZ. Shoaling of the AMZ due to global warming could thus lead to more extensive areas with high rates of SCM biological activity, with the diel oxic/anoxic cycles of these SCMs influencing marine productivity and coupled global nitrogen cycling.

Materials and Methods

Sampling Sites and in Situ Measurements. The two main oxygen minimum zones of the ETSP and ETNP were investigated during two cruises during 2014: the Activities of Research Dedicated to the Minimum of Oxygen in the Eastern Pacific (AMOP) cruise on the RV *L'Atalante* to the ETSP off Peru during late January and February 2014 and the Oxygen Minimum Zone Microbial Biogeochemistry Expedition 2 (OMZOMBIE2) cruise on the RV *New Horizon* to the ETNP region off Mexico during May–June 2014. Profiles of physical and chemical variables were obtained with a Seabird SBE-911 CTD system, equipped with a SBE 43 oxygen sensor and a Seapoint Chlorophyll Fluorimeter (RV *New Horizon*) or a Chelsea Aqua 3 fluorimeter (RV *L'Atalante*). CTD sensors were calibrated according to the manufacturer. The fluorometers used for the determination of chlorophyll were calibrated using pure chlorophyll solutions in 90% acetone (from 0.1 to 100 µg/L). In the ETNP, a pump profiling system (PPS) was also used for water collection. High-resolution O₂ profiling was performed during the CTD and PPS casts during the ETNP cruise. A high-resolution STOX sensor (2, 28) was used to measure O₂ concentration at nanomolar levels as described previously (2, 4).

Flow Cytometry Analysis. Samples for cell counts were taken at several depths from the rosette (ETNP and ETSP) and the PPS (ETNP), fixed with glutaraldehyde and stored at –80 °C until analysis. Cell abundance was determined by flow cytometry using a FACSCalibur flow cytometer (Beckton Dickinson). *Prochlorococcus*, *Synechococcus*, and other autofluorescent cells (identified as picoeukaryotes) were counted in untreated samples, whereas autofluorescent plus nonautofluorescent cells (bacteria + archaea, referred as total microbial

community) were analyzed by staining the cells with SYBR Green (Molecular Probes) as described previously (29, 30).

Oxygen Production and Carbon Fixation Measurements. Water samples from the SCM (summarized in Table S1) were collected using Niskin bottles or a PPS. To minimize the O₂ leaking from the polymers of the Niskin bottles, the water was transferred to a 20-L glass bottle previously purged with N₂ gas as soon as the rosette was on deck. If the samples were collected using the PPS, the 20-L glass bottle purged with N₂ gas was filled directly from the outlet of the PPS. A certain O₂ contamination (1–5 µmol·L⁻¹) during the sampling procedure could not be avoided, and the seawater was therefore immediately degassed in the 20-L bottle by bubbling with N₂ + 0.05% CO₂. A STOX sensor was inserted inside the bottle to determine when anoxia was approached (<100 nmol O₂·L⁻¹). After adjusting the O₂ concentration to 100–400 nmol·L⁻¹, samples were siphoned to custom made incubation vessels ($n = 12–16$) (Fig. S5) (31, 32), containing either STOX sensors (ETSP) or a combination of STOX and optode sensors with a measuring range of 0–1,000 nmol·L⁻¹ (32, 33) (ETNP). Each vessel was placed inside a light incubation tube immersed in a constant temperature water bath, enabling maintenance of in situ temperature (14–15 °C) and quantification of very low O₂ transformation rates. The light incubation tubes consisted of a black PVC tube with white LEDs (LF065-W3F-850; OSRAM) installed along the whole periphery of the tube and with a custom-built waterproof magnetic stirrer fitted at the bottom. The LEDs were covered with a blue filter (131 Marine Blue filter; LEE Filters) to simulate the in situ light spectrum. Oxygen concentrations (Fig. 2) throughout the incubation period (8–12 h) were measured in treatments spanning a range of bluish light intensities slightly above maximum in situ levels (10, 20, and 40 µmol photons·m⁻²·s⁻¹) and in darkness ($n = 3–4$, per treatment). Rates of oxygen consumption or production (here named NCP) were obtained by linear regression of the oxygen evolution during the incubations. GCP rates were calculated by subtracting the mean respiration value (NCP rate measured in darkness) from the NCP rates measured at different irradiances.

Rates of carbon incorporation were measured simultaneously during the incubations for oxygen measurements using stable (ETNP) or radioactive (ETSP) isotopes. Incubations amended with Na¹⁴C-HCO₃ (450 µCi/L final concentration) were done in parallel incubation bottles of only 110 mL (but otherwise similar to the one described in Fig. S5) following the procedure described by Telling et al. (34). Incubations amended with Na¹³C-HCO₃ (0.27 mM ¹³C final concentration) were done in the same incubation bottles used for O₂ measurements. Incorporation of ¹⁴C was measured by counting on a Perkin Elmer Tri-Carb 2900 TR scintillation counter, whereas the ¹³C incorporation was analyzed in an Elemental Analyzer (Thermo Elemental Analyzer Flash EA 1112 HT) coupled to an Isotope Ratio Mass Spectrometer (Delta V; Thermo Scientific). The ¹³C enrichment in the produced organic carbon was calculated as the difference between the amounts of ¹³C in the sample minus the natural ¹³C abundance measured on blank filters. Decays per minute values (¹⁴C incubations) and ¹³C incorporation were converted to ¹²C uptake values or GCP (nmol C·L⁻¹·h⁻¹) rates using the formula described in Telling et al. (34).

Rates Modeling and Upscaling of Processes. The photosynthesis–irradiance model described by Jassby and Platt (35) was fitted to the measured GCP (nmol·L⁻¹·h⁻¹) rates for both O₂ production and C assimilation (Fig. 2), being

$$\text{GCP} = \text{GCP}_{\text{max}} \times \tanh(\alpha \times E / \text{GCP}_{\text{max}}),$$

where GCP_{max} (nmol·L⁻¹·h⁻¹) is the maximum gross community production rate, reached at saturating irradiances, tanh is the hyperbolic tangent, α [(nmol·L⁻¹·h⁻¹)(µmol photons·m⁻²·s⁻¹)⁻¹] is an index of the photosynthetic efficiency, and E (µmol photons·m⁻²·s⁻¹) is the spherical irradiance.

The obtained parameters were normalized by the chlorophyll concentration and used to estimate the in situ O₂ production and C fixation using the light and chlorophyll profiles measured in the SCM by the fluorescence and photosynthetic active radiation (PAR) sensors connected to the CTD (ETSP cruise) or the PPS (ETNP cruise). During the ETSP cruise off Peru, casts were consistently repeated every 3–4 h, and thus, the light profiles from the CTD were used in our calculations. During the ETNP cruise, light profiles measured with the PPS during daytime were normalized to the incident irradiance at the surface. The light attenuation profiles were assumed to be constant at each station, and the incident irradiance was used to calculate the change in light profile during the day. The values of incident irradiance were taken from the closest National Radiation station located in San Diego (National Solar Radiation Database, National Oceanic and Atmospheric Administration, United States).

Metatranscriptome Analysis. Community cDNA sequencing was used to characterize microbial gene transcription in biomass (retained on 0.22- μ m filters) from a subset of AMZ samples at the ETNP region off Mexico (Tables S4 and S5). These included samples collected during the OMZOMBiE2 cruise (2014) and a subset of samples previously reported by Padilla et al. (36). Seawater from discrete depths spanning the oxic zone, SCM, lower oxycline, upper AMZ, and AMZ core was collected using Niskin bottles or the PPS. The sampling, preservation, RNA extraction, and sequencing were done following the procedure described by Padilla et al. (36). Barcoded sequences were demultiplexed, and low-quality reads (Phred score < 25) were removed. Paired-end sequences were merged using custom scripts incorporating the FASTX toolkit (hannonlab.cshl.edu/fastx_toolkit/index.html) and USEARCH algorithm, with criteria of minimum 10% overlap and 95% nucleotide identity within the overlapping region. Ribosomal RNA (rRNA) transcripts were identified with riboPicker (37) and removed from the analysis. Merged nonrRNA sequences were queried via DIAMOND using sensitive search parameters (38) against the National Center for Biotechnology Information (NCBI)-nr database (November 2013). DIAMOND-identified protein-coding transcripts were assigned a functional annotation based Kyoto Encyclopedia of Genes and Genomes (KEGG) orthology (KO) identifiers (39) using Metagenome Analyzer 5 (MEGAN5) (40), with taxonomic classification assigned using the lowest common ancestor (LCA) algorithm in MEGAN5 based on the NCBI taxonomy. Counts per KO were normalized to the total number of protein coding transcripts classified within bacteria and archaea (i.e., prokaryotes). Transcripts encoding LATO and HATO, *nrx*, and *amo* (all subunits) were identified by the KO identifiers listed in Table S4.

NOB abundances were determined by taxonomic LCA assignment according to NCBI taxonomy of DIAMOND-identified mRNA transcripts normalized to the total number of prokaryotic mRNA sequences. Taxonomic affiliation of both LATO and HATO were also assigned according to NCBI taxonomy via the LCA algorithm in MEGAN5.

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