Dark biological superoxide production as a significant flux and sink of marine dissolved oxygen

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January 3, 2020 (received for review July 19, 2019)

The balance between sources and sinks of molecular oxygen in the oceans has greatly impacted the composition of Earth’s atmosphere since the evolution of oxygenic photosynthesis, thereby exerting key influence on Earth’s climate and the redox state of (sub)surface Earth. The canonical source and sink terms of the marine oxygen budget include photosynthesis, respiration, photosynthesis, the Mehler reaction, and other smaller terms. However, recent advances in understanding cryptic oxygen cycling, namely the ubiquitous one-electron reduction of O2 to superoxide by microorganisms outside the cell, remains unexplored as a potential player in global oxygen dynamics. Here we show that dark extracellular superoxide production by marine microbes represents a previously unconsidered global oxygen flux and sink comparable in magnitude to other key terms. We estimate that extracellular superoxide production represents a gross oxygen sink comprising about a third of marine gross production, and a net oxygen sink amounting to 15 to 50% of that. We further demonstrate that this total marine dark extracellular superoxide flux is consistent with concentrations of superoxide in marine environments. These findings underscore prolific marine sources of reactive oxygen species and a complex and dynamic oxygen cycle in which oxygen consumption and corresponding carbon oxidation are not necessarily confined to cell membranes or exclusively related to respiration. This revised model of the marine oxygen cycle will ultimately allow for greater reconciliation among estimates of primary production and respiration and a greater mechanistic understanding of redox cycling in the ocean.

Significance

Extracellular production of the reactive oxygen species (ROS) superoxide results from the one-electron reduction of O2. Nearly all major groups of marine microbes produce extracellular superoxide. In this global estimate of marine microbial superoxide production we determine that dark extracellular superoxide production is ultimately a net sink of dissolved oxygen comparable in magnitude to other major terms in the marine oxygen cycle. This abundant source of superoxide to the marine water column provides evidence that extracellular ROS play a significant role in carbon oxidation and the redox cycling of metals in marine environments. Consideration of this significant reductive flux of dissolved oxygen is essential for field, laboratory, and modeling techniques for determining productivity and oxygen utilization in marine systems.

Author contributions: K.M.S., S.D.W., and C.M.H. designed research; K.M.S. performed research; K.M.S. analyzed data; and K.M.S., S.D.W., and C.M.H. wrote the paper.

The authors declare no competing interest.

This article is a PNAS Direct Submission.

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extracellular fluxes (9, 23, 24). Superoxide has a half-life on the order of a few minutes or less (9, 25), with its fate highly dependent on seawater chemistry; superoxide may be reoxidized to O₂ or reduced to H₂O₂, other peroxides, and ultimately water by reactions with redox-active metals including copper, iron, and manganese, organic matter, or via (un)catalyzed dismutation (26, 27).

Extracellular superoxide plays a diverse suite of roles in cell physiology, including cell signaling, growth, defense, and redox homeostasis. In microbial eukaryotes, enzymatic extracellular superoxide production is involved in cell differentiation (19). In plants, superoxide serves as a pretranscription defense in wound repair (16). Recently, extracellular superoxide within marine bacteria was shown to be tightly regulated through the growth cycle with superoxide eliminated upon entering stationary phase (28). Addition of superoxide dismutase (SOD), an enzyme that eliminates superoxide, inhibited growth, suggesting that superoxide is involved in growth and proliferation as previously suggested for pathogenic strains of *Escherichia coli* and *Chattonella marina* (29–32). Further, recent evidence indicates that extracellular superoxide production by the diatom *Thalassiosira oceanica* may play a critical role in maintaining the internal redox conditions in photosynthesizing cells (21). This suite of beneficial physiological processes all result from or result in the reduction of molecular oxygen that is not otherwise considered in biogeochemical cycles of oxygen and related elements.

Our aim in this study is to leverage recent assessments of dark extracellular superoxide production rates by globally important groups of marine microbes to determine the resulting oxygen sink. Our approach consists of two parts: 1) constrain the gross oxygen reduction that results from dark, extracellular superoxide production and 2) estimate the fraction of gross superoxide produced that is ultimately reduced to water. Generally speaking, the most abundant group of organisms in the global ocean is heterotrophic bacteria. Alphaproteobacteria and Gammaproteobacteria comprise >70% of heterotrophic bacteria in the water column, and a subset of Alphaproteobacteria, Pelagibacteria (SAR11 clade), makes up nearly 25% of cells in the ocean (33, 34). Here we compile measured extracellular superoxide production rates of several marine Alphaproteobacteria and Gammaproteobacteria, including SAR11 clade organisms (Fig. 1). Although far outnumbered by marine heterotrophs in the global ocean, oxygenic phototrophs have been shown to produce both light-dependent and -independent extracellular superoxide up to several orders of magnitude higher than average marine heterotrophs on a per-cell basis, meaning a representative accounting of the superoxide flux from phototrophs is necessary to adequately constrain superoxide production (9, 13, 35). In this study we include superoxide production rates of the following organisms: *Prochlorococcus* and *Synechococcus*, the two most abundant photosynthetic organisms in the global ocean; *Trichodesmium*, a dominant N₂-fixing cyanobacterium in oligotrophic waters; coccolithophores, the most abundant group of calcifying microalgae present throughout the global ocean; several species of diatoms, a diverse group of silicifying algae found in nutrient rich waters; and *Phaeocystis*, an alga predominantly found in the Southern Ocean. In constructing this estimate of superoxide production, we assign measured dark superoxide production rates to organisms that fall within these groups of organisms and conservatively consider all others to be nonproducing.

Marine heterotrophs belonging to Alphaproteobacteria and Gammaproteobacteria, including two Pelagibacterales members, produce gross extracellular superoxide within a fairly narrow range of 0.1 to 3.7 amol-cell⁻¹-h⁻¹ (Fig. 1). Oxygenic phototrophs, with the exception of *Prochlorococcus*, far exceed heterotrophic cell-normalized superoxide production, with net production rates ranging from 4.3 to 13,400 amol-cell⁻¹-h⁻¹ (17). *Prochlorococcus* produces significantly less extracellular superoxide across four ecotypes, with average gross rates ranging from 0.007 to 0.091 amol-cell⁻¹-h⁻¹ (17). Also shown in Fig. 1 is an estimate for the average marine cell O₂ utilization rate assuming a balanced marine oxygen budget (see SI Appendix for calculation), or in other words the total amount of oxygen produced in the global ocean, divided by the number of cells. This estimate demonstrates that dark extracellular superoxide production of several groups of marine organisms exceeds the average oxygen utilization rate. Here we use the more general term “oxygen utilization” instead of “respiration” because other oxygen-consuming biological reactions (e.g., photorespiration)

Fig. 1. Extracellular superoxide production rates. Cyanobacteria (green) include *Synechococcus*, *Trichodesmium*, and *Prochlorococcus*; eukaryotic algae (orange) include coccolithophores, diatoms, and *Phaeocystis*; and heterotrophic bacteria (blue) include Pelagibacteria (SAR11 clade), other Alphaproteobacteria, and Gammaproteobacteria (see SI Appendix for data, sources, and organism details and SI Appendix, Table S1 for diatom rates). Average marine cell oxygen consumption rate is shown in gray (SI Appendix).
and the Mehler reaction) are each responsible for >10% of the marine oxygen sink. To extrapolate these superoxide production values to the global ocean, we provide estimates of total cell numbers in the water column of each organismal group included in this study (Table 1). We assigned cells from each group the mean and standard error (SE) determined from our bootstrapping approach and used a Monte Carlo approach to determine the mean and probability distribution for the whole ocean superoxide flux. A slightly different approach was used for diatoms (Methods). Using these total cell number estimates and modeled dark extracellular superoxide production rates for each organismal group, we calculate that gross dark extracellular superoxide production represents a flux of $3.9 \pm 0.5 \times 10^{15}$ mol O$_2$ per year. For comparison, a central estimate for GOP in all marine environments derived from estimates of marine productivity is $1.09 \times 10^{16}$ mol O$_2$ y$^{-1}$ (SI Appendix) (36). Thus, gross light-independent extracellular superoxide production by microorganisms represents an O$_2$ loss flux ~36 (±5)% of marine GOP. This gross superoxide production estimate is illustrative for demonstrating the size of this reductive flux; however, it is the net reduction of superoxide that ultimately determines the weight of this reductive flux on the global oxygen cycle. We provide constraints on the net reduction of extracellular superoxide below.

While we show that model estimates based on laboratory-based rates yield a dark superoxide flux that is a substantial and previously unrecognized part of the global oxygen budget, a claim that represents such a significant shift in the model of marine oxygen utilization requires some ground truthing with environmental data. In particular, we used a bootstrapping approach to estimate the mean and SE of all extracellular superoxide production rates available in the literature for each group discussed here (SI Appendix, Fig. S1). The available data on extracellular superoxide production within axenic cultures do not contain the ideal richness for relying on this numerical approach alone. Thus, we tested our culture-based estimate by calculating expected marine superoxide concentrations based on our estimate of global superoxide production and compared these values to available marine superoxide concentration data. If our estimate for average marine superoxide concentration falls within observations, this provides an independent line of evidence that extracellular superoxide production comprises a significant global oxygen flux, with our reasoning as follows. The mean pseudo-first-order decay rate constant of superoxide in marine environments has been previously characterized at 0.0106 s$^{-1}$, with a 1σ confidence interval from 0.0050 s$^{-1}$ to 0.0226 s$^{-1}$ (SI Appendix, Fig. S3) (35, 37–39). Using these observed decay rate constants we estimate steady-state superoxide concentrations from biological production to be 152 pM (1σ confidence interval: 71 to 322 pM) in the surface ocean and 0.6 pM (1σ confidence interval: 0.3 to 1.3 pM) below 200 m (SI Appendix, Table S2). All available superoxide concentration measurements from the marine water column are shown in Fig. 2 along with the calculated expected concentration range based on our model of global superoxide production (gray bar). As indicated, the expected range of superoxide concentrations is largely consistent with measurements collected in the surface ocean, and, in fact, underestimates measured concentrations in all deep ocean measurements. Although not shown in Fig. 2, superoxide concentrations in some coastal systems

Table 1. Estimate of global superoxide flux: Summary of cell number estimates, cell specific superoxide production rates, and contribution of each marine clade toward the marine superoxide flux

| Group            | Cell estimate* | Range superoxide production, amol-cell$^{-1}$·h$^{-1}$ | Mean model value*, amol-cell$^{-1}$·h$^{-1}$ | Superoxide production, mol y$^{-1}$ |
|------------------|----------------|--------------------------------------------------------|------------------------------------------|-----------------------------------|
| Synechococcus    | $7.0 \times 10^{26}$ | 4.3–550                                                | 106                                       | $6.49 \times 10^{14}$            |
| Trichodesmium    | $4.6 \times 10^{23}$ | ND–1,500                                               | 465                                       | $1.87 \times 10^{12}$           |
| Prochlorococcus  | $2.9 \times 10^{27}$ | 0.007–0.091                                            | 0.06                                      | $1.40 \times 10^{12}$           |
| Coccolithophores | $2.6 \times 10^{26}$ | ND–5,300                                               | 342                                       | $7.78 \times 10^{12}$           |
| Diatoms          | $2.6 \times 10^{24}$ | 75–13,000                                              | 47,560$^\dagger$                          | $1.07 \times 10^{15}$           |
| Phaeocystis      | $3.9 \times 10^{25}$ | 1,700–4,200                                            | 2,925                                     | $9.99 \times 10^{14}$           |
| Pelagibacterales (SAR11) | $2.4 \times 10^{28}$ | 0.11–0.23                                              | 0.15                                      | $3.20 \times 10^{13}$           |
| Alphaproteobacteria (without SAR11) | $1.9 \times 10^{28}$ | 0.1–3.1                                               | 0.9                                       | $1.51 \times 10^{14}$           |
| Gammaproteobacteria | $2.7 \times 10^{28}$ | 0.1–3.7                                               | 1.1                                       | $2.57 \times 10^{14}$           |
| Total            |                |                                                        |                                           | $3.94 \times 10^{15}$           |

*See SI Appendix for sources, derivations of cell number estimates, and model value assignment.

†Calculated using cell surface area normalized rates; see SI Appendix.

Fig. 2. Measured and expected marine superoxide concentration. Compiled marine superoxide measurements from Rose et al. (35) (green diamond), Hansard et al. (38) (yellow triangle), Rusak et al. (39) (red square), and Roe et al. (37) (blue circle). The gray bars indicate the 68% confidence interval: 0.3 to 1.3 pM below 200 m (SI Appendix, Table S2). All available superoxide concentration measurements from the marine water column are shown in Fig. 2 along with the calculated expected concentration range based on our model of global superoxide production (gray bar). As indicated, the expected range of superoxide concentrations is largely consistent with measurements collected in the surface ocean, and, in fact, underestimates measured concentrations in all deep ocean measurements. Although not shown in Fig. 2, superoxide concentrations in some coastal systems
have been reported as high as 120 nM, four orders of magnitude higher than our estimate, underscoring the conservative nature of our estimate (40).

**Additional Sources of Superoxide**

This accounting of the marine superoxide flux only considers extracellular dark superoxide production by dominant organisms with known production rates. In fact, the total O$_2$ flux via all superoxide production pathways in marine environments undoubtedly exceeds our estimates here by a sizable margin and would lead to an even greater contribution of superoxide production on O$_2$ loss. In particular, light-dependent (a)biotic superoxide production is not included in our present estimate. Within sunlit waters, there is significant abiotic photochemical production of superoxide, and extracellular superoxide production rates by marine phototrophs are significantly higher in the light (13, 41, 42). Indeed, the extracellular superoxide production exhibited by multiple species of diatoms more than doubled in the presence of light (42). The same behavior was observed for the coccolithophore *Emiliania Huxleyi* (18) and certain *Trichodesmium* ecotypes (13), where light-dependent increases in extracellular superoxide could not be accounted for by abiotic factors. Since phototrophs as a group are responsible for most of the dark extracellular superoxide production, even modest modulation in extracellular superoxide production in the light could produce a substantial increase in our estimate of the gross superoxide flux and the net oxygen sink that results, which we discuss in the next section.

Possibly the best-characterized abiotic source of superoxide in the oceans is the photochemical excitation of chromophoric dissolved organic matter (CDOM) and subsequent reduction of O$_2$ to superoxide in the surface ocean (43). One model using a remote sensing approach to estimate photochemical reactive oxygen species (ROS) production in the surface ocean estimated that midday superoxide concentrations resulting from photochemical production and a range of superoxide sinks ranged from near 10 pM at high latitudes to near 200 pM at low latitudes (41). These results demonstrate that photochemical production of superoxide can exceed dark biological production in sunlit surface waters. Notably, contribution of this photochemical superoxide will vary temporally and decrease with depth upon the attenuation of photoactive wavelengths (290 to 490 nm), with local variations in productivity and the compositional nature of surface ocean CDOM also impacting its reactivity.

**The Fate of Superoxide**

Both laboratory- and field-based measurements converge on the similar conclusion that dark extracellular superoxide flux is a substantial component of oxygen turnover in the global ocean. To further place this process into the context of the global O$_2$ budget, it is important to distinguish gross dark superoxide production from the net loss of dissolved O$_2$. Superoxide in aqueous systems may decay by oxidation back to O$_2$ with no net effect on marine dissolved oxygen, or via reduction, which may lead to a net loss of oxygen. Superoxide decay is considered the primary source of hydrogen peroxide (H$_2$O$_2$), and thus much of what we know and assume about the fate of superoxide is inferred from studies of hydrogen peroxide concentration and rate measurements. Nevertheless, other secondary and direct sources of hydrogen peroxide have also been identified within microbial cultures (42, 44, 45) and natural waters (10). For the purposes of this estimate, we accept the general premise that superoxide decay is the primary source of hydrogen peroxide and ignore other sources, while recognizing that the foundation of this premise needs further evaluation. It is also often assumed that the primary reduction or disproportionation product of superoxide production is H$_2$O$_2$; however, superoxide decay may also occur through bond-forming redox reactions with dissolved organic carbon, metals, or through unknown sinks (46–50). Such reactions will not produce an H$_2$O$_2$ intermediate. In fact, it has been shown that hydrogen peroxide formation can only account for 45% of net oxygen loss in photochemical oxidation of marine waters (50). While photochemical superoxide production is not a perfect analogy for dark biological superoxide production, the large body of work investigating the fate of photochemically derived superoxide offers the most transferable insight into the likely fate of biologically derived superoxide in seawater. What follows is our attempt to constrain the net reduction of marine dissolved oxygen from the estimate of gross extracellular superoxide production using a combination of measurements from photochemical and biogeochemical literature.

Ultimately, the fate of superoxide and hydrogen peroxide is highly dependent on the availability of dissolved organic matter (and its reactivity), the abundance of redox-active metals (and their redox states), and the expression of enzymes that eliminate ROS such as SOD, catalase, and peroxidases (49, 51, 52). Superoxide can be both oxidized (Eq. 1) and reduced (Eq. 2), leading to the net consumption of 0 or 1 mol O$_2$ per mole superoxide, respectively (Fig. 3). Superoxide can also undergo uncatalyzed dismutation, a process that results in 50% oxidation to O$_2$ and 50% reduction to H$_2$O$_2$ (Eq. 3). Hydrogen peroxide is more stable than superoxide in natural waters, with pseudo-first-order decay rate constants approximately three orders of magnitude lower than that of superoxide. Consequently, H$_2$O$_2$ has a lifetime of ~1 to 2 d, and typical concentrations are ~10$^3$ higher for H$_2$O$_2$ than for superoxide [from <1 nM in the deep ocean to ~100 nM in sunlight surface water (53, 54)]. Typical production and decay rates range from 0.8 to 2.4 nM h$^{-1}$ for dark seawater (53) and 0.9 to 8.3 nM h$^{-1}$ in sunlit seawater (55). As for the fate of H$_2$O$_2$, previous work has shown that light-independent, biological processes are primarily responsible for its degradation in marine systems, with 65 to 80% of H$_2$O$_2$ degradation resulting from catalase activity (Eq. 4) and the remainder resulting from peroxidase activity (Eq. 5) (53):

$$O_2^\cdot + e^- \rightarrow O_2 \quad \text{(oxidation)}$$  \[1\]

$$O_2^\cdot + 2H^+ + e^- \rightarrow H_2O_2 \quad \text{(reduction)}$$  \[2\]

$$2O_2^\cdot + 2H^+ \rightarrow H_2O_2 + O_2 \quad \text{(SOD or uncatalyzed dismutation)}$$  \[3\]

$$2H_2O_2 \rightarrow 2H_2O + O_2 \quad \text{(catalase-based H}_2O_2 \text{ decay)}$$  \[4\]

$$H_2O_2 + AH_2 \rightarrow 2H_2O + A \quad \text{(peroxidase-based H}_2O_2 \text{ decay)} \quad \text{[5]}$$

Assuming this range of catalase and peroxidase activity holds throughout the water column, between 60 and 67.5% of H$_2$O$_2$ is
Ultimately reduced to water. Thus, the theoretical net loss of oxygen through the superoxide production pathway can range from 0 (e.g., all superoxide is oxidized back to O₂; Eq. 1) to 67.5% (e.g., superoxide reduction to H₂O₂; Eq. 2), followed by catalase- and peroxidase-mediated degradation of H₂O₂ (Eqs. 4 and 5) (Fig. 3).

There are two ways we can use previous data to estimate the net sink of oxygen that results from the dark biological superoxide flux in the ocean. The first, and more conservative, approach is to multiply the global superoxide production flux by the ratio of hydrogen peroxide production to superoxide production observed in natural waters, P̂₁₂O₂/P̂O₂, followed by the oxidation–reduction ratio observed for hydrogen peroxide. This yields the total O₂ reduced to water via an H₂O₂ intermediate and accounts for dismutation (Eq. 3), univalent oxidation (Eq. 1), and univalent reduction (Eq. 2). This method will produce a more conservative estimate of net oxygen loss because it implicitly assumes that all superoxide not reduced to hydrogen peroxide is reoxidized to O₂, when in reality a large fraction of the superoxide sink may yield products other than hydrogen peroxide (e.g., organic peroxides or other ROS) (49, 50). The second approach uses the same P̂₁₂O₂/P̂O₂ ratio to determine the hydrogen peroxide flux that results from the superoxide flux, multiplied by the observed ratio of net oxygen loss to hydrogen peroxide. This approach assumes that a marine photochemical system where superoxide is implicated as the primary oxidant. This approach may slightly overestimate the net oxygen sink because it ignores the possibility that some oxygen reduction may occur through multitranfer transfers (50).

For both of these approaches, we need a reasonable estimate of the production ratio of hydrogen peroxide to that of superoxide (P̂₁₂O₂/P̂O₂). In experiments investigating production of superoxide and hydrogen peroxide during DOM irradiation, P̂₁₂O₂/P̂O₂ ranged from 0.10 to 0.67, with an average value of 0.24 (55). Another similar study on waters collected from the transition between terrestrial and marine environment found that P̂₁₂O₂/P̂O₂ ranged from 0.5 in riverine waters to 0.30 in Gulf Stream waters (56). Slightly lower values (0.08 to 0.17) have been observed in photochemical systems with terrestrial organic matter (57), possibly due to the differing nature of terrestrial DOM (aromatic vs. aliphatic). In a brackish and a freshwater pond, P̂₁₂O₂/P̂O₂ sometimes exceeded the stoichiometry of 0.5 expected for dismutation, suggesting there may be other sources of hydrogen peroxide (10). We use the average value presented by Powers et al. (55) of 0.24 as the most relevant to the marine environment and the best choice at this time for a global marine estimate. Using our first approach and P̂₁₂O₂/P̂O₂ of 0.24, we estimate a lower bound for the net oxygen reduction resulting from superoxide production at 14.4% of the marine superoxide flux (or 5% of the marine oxygen budget). A significant fraction of this superoxide (52% of the marine superoxide flux) is unaccounted for because it does not produce hydrogen peroxide, meaning the fate of as much as 19% of the marine oxygen budget that cycles through superoxide remains unknown.

Using the second approach, we assume hydrogen peroxide formation and net oxygen loss that result from superoxide formation occur with a fixed ratio in seawater [0.45:1 H₂O₂:O₂] (50). We find that superoxide production will result in a net oxygen loss of 53% of the global superoxide flux (or 19% of the marine oxygen budget).

These two estimation methods suggest dark, biological production of extracellular superoxide is a sink between 5 and 19% in the marine oxygen budget, indicating that the oxygen sink from dark extracellular superoxide production is similar in magnitude to the Mehler reaction and photosorption. Notably, total superoxide production in photochemical systems has been shown to correlate with CO₂ production from DOM (58), suggesting that a significant fraction of the superoxide sink is ultimately through reduction tied to organic carbon oxidation, favoring the higher end of this estimate. Shipboard incubations show that marine superoxide decay is primarily correlated with dissolved organic matter and dissolved manganese, both of which allow for a significant reductive sink of superoxide (26, 51) and subsequent higher O₂ loss. Oxygen loss in marine environments has previously been attributed primarily to a combination of respiration, photorespiration, and the Mehler reaction in the surface ocean and respiration alone in the deep ocean. We propose that dark extracellular superoxide production and its net oxygen sink has likely been overlooked and unintentionally incorporated into other sinks because separation of this secondary physiological process presents a methodological challenge. The sinks of marine oxygen resulting from respiration, photorespiration, and the Mehler reaction should therefore be revised downward to accommodate for the reductive sink from extracellular superoxide production.

Further Implications

We anticipate that the identification of an appreciable cryptic sink of oxygen, and consequently organic carbon, may help reconcile spatiotemporal and methodological discrepancies among measurements of marine primary productivity arising as variable contributions of superoxide production to net O₂ loss. Adding another layer of complexity, the resulting H₂O₂ produced in excess during times of increased metabolic activity (e.g., photosynthesis), may be stored in extracellular organics under light or dark storage, persisting in the environment for hours to days. These processes may lead to heterogeneous O:C reaction stoichiometry as well as possible complexity in corresponding stable isotope dynamics. As measurements of primary productivity collected using multiple methods concurrently can produce primary productivity estimates that vary more than an order of magnitude (59), we propose that some ROS cycling and ROS-related oxygen loss may explain some of these discrepancies (e.g., ¹⁴C vs. ¹³C). Production and consumption of extracellular superoxide (coupled with the oxidation of intracellular reducing equivalents) may lead to differential redox evolution of marine carbon reservoirs that are spatially or functionally separated (e.g., surface vs. deep, particulate vs. dissolved). While superoxide production will not affect the whole ocean –O₂:C, it may drastically influence the amount and rate of organic carbon that is directly remineralized to CO₂ vs. that which proceeds through partially oxidized dissolved organic compounds. The exact utility of extracellular superoxide for cells and the magnitude of influence superoxide has on the global carbon budget (today and throughout Earth history) both remain important and open questions. Nevertheless, this newly identified gross flux of superoxide in the global ocean underscores the critical role ROS play in the global cycling of O₂, carbon, and redox active elements essential to life.

Methods

The dark superoxide production rates compiled in this study, with the exception of coccolithophores, were measured from cells grown to mid-exponential phase under ideal growth conditions using a flow-injection chemiluminescent approach (9). The study investigating the cell-specific superoxide production rate of coccolithophores measured their production rate throughout their growth curve, which we converted to a time-weighted average using trapezoidal integration of the cell superoxide production rate as a function of time (18). Cell-normalized superoxide production rates presented in the scientific literature are either presented as net or gross superoxide production rates, the latter requiring an exogenous spike of superoxide to determine the proportion of extracellular ROS that is enzymatically degraded by the organisms. Gross superoxide production rates
were used where available; otherwise, net superoxide production rates were used. All compiled rates are presented in Fig. 1. Bootstrap resampling of net superoxide production rates was conducted to estimate the rate distribution of each organism group (SI Appendix, Fig. S1), with the exception of diatoms. Diatom production rate was normalized to cell surface area and diatom surface area was determined from a database containing >90,000 georeferenced diatom observations (SI Appendix, Table S1). Cell abundances for phototroph and heterotroph groups included in this study were compiled or calculated from available literature data and are discussed in greater detail in Methods. We note that when net primary productivity (NPP) estimates were used to estimate cell abundances, each group’s fractional contribution to NPP (as opposed to moles of carbon fixed) was given preferance. Fraction contribution to NPP was preferred because it allows for direct comparison between GPP and total superoxide production in a way that minimizes the influence of differences in productivity estimates between studies. Total superoxide flux was estimated using a Monte Carlo approach incorporating superoxide production rate distribution for each organism group. Parameter choices and results are presented in SI Appendix, Estimation of Global Superoxide Flux. Superoxide concentrations and uncertainties were estimated using the volume-normalized superoxide flux and compilation of superoxide decay rates from the literature (SI Appendix, Fig. S3 and Table S2). Further experimental methods are described in SI Appendix. All data compiled and generated in this study are provided within the main text and SI Appendix.

ACKNOWLEDGMENTS. This work was supported by NASA Earth and Space Science Fellowship NNX15AR62H to K.M.S., NASA Exobiology grant NNX15AM04G to S.D.W. and C.M.H., and NSF Division of Ocean Sciences grant 1355720 to C.M.H. This research was further supported in part by Hanse-Wissenschaftsakademie Institute of Advanced Study fellowships to C.M.H. and S.D.W. We thank Danielle Hicks for assistance with figures and Community Earth Systems Model (CESM) Large Ensemble Project for the availability and use of its data product. The CESM project is primarily supported by the NSF.

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