A theoretical basis for a nanomolar critical oxygen concentration

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Abstract

When aerobic microbes deplete oxygen sufficiently, anaerobic metabolisms activate, driving losses of fixed nitrogen from marine oxygen minimum zones. Biogeochemical models commonly prescribe a 1–10 μM critical oxygen concentration for this transition, a range consistent with previous empirical and recent theoretical work. However, the recently developed STOX sensor has revealed large regions with much lower oxygen concentrations, at or below its 1–10 nM detection limit. Here, we develop a simplified metabolic model of an aerobic microbe to provide a theoretical interpretation of this observed depletion. We frame the threshold as O_2^*, the subsistence oxygen concentration of an aerobic microbial metabolism, at which anaerobic metabolisms can coexist with or outcompete aerobic growth. The framework predicts that this minimum oxygen concentration varies with environmental and physiological factors and is in the nanomolar range for most marine environments, consistent with observed concentrations. Using observed grazing rates to calibrate the model, we predict a minimum oxygen concentration of order 0.1–10 nM in the core of a coastal anoxic zone. We also present an argument for why anammox may be energetically favorable at a higher oxygen concentration than denitrification, as some observations suggest. The model generates hypotheses that could be tested in the field and provides a simple, mechanistic, and dynamic parameterization of oxygen depletion for biogeochemical models, without prescription of a fixed critical oxygen concentration.

Anaerobic processes in marine oxygen minimum zones (OMZs) are one of the major loss pathways for fixed nitrogen in the ocean (Ward 2013). With predicted marine deoxygenation and the open question of whether or not OMZs may expand due to global warming (IPCC 2014), establishing theory for the controls on aerobic vs. anaerobic processes is timely. Qualitatively, the mechanisms that form OMZs and lead to fixed nitrogen loss are well understood: in productive areas of the ocean, enhanced aerobic respiration in poorly ventilated subsurface waters depletes oxygen (Devol 2008). When oxygen is sufficiently low, anaerobic metabolisms become energetically competitive pathways, resulting in the accumulation of metabolic products such as nitrogen gas (N_2) and nitrous oxide (N_2O) (Devol 2008; Ulloa et al. 2012; Wright et al. 2012). In OMZs, two pathways—heterotrophic denitrification and chemoautotrophic anaerobic ammonium oxidation (anammox)—account for the majority of fixed nitrogen loss (Ward 2013).

Studies of microbial processes in aquatic oxygen minimum zones have revealed complex biogeochemical habitats (Lam and Kuypers 2011; Wright et al. 2012). Microbial community composition exhibits structure along the oxygen gradient between end-member fully oxic and fully sulfidic environments (Gonsalves et al. 2011; Ulloa et al. 2012; Jayakumar et al. 2013; Hawley et al. 2014). In the oxycline, as oxygen sharply depletes by up to five orders of magnitude (Fig. 1), microbial communities have been observed to be more diverse than in the anoxic cores (Jayakumar et al. 2009; Zaikova et al. 2010; Bryant et al. 2012), although not always (Stevens and Ulloa 2008). Organic matter supply to the subsurface that varies in time and space creates a dynamic oxycline (Ward et al. 2008), which may support this diversity, with competitive exclusion operating progressively with depth as environmental conditions stabilize (Hutchinson 1961).

Devol (1978) noted that predicting the oxygen concentration of the switch between aerobic and anaerobic respiration is crucial for accurate OMZ modeling. He conducted an exhaustion curve experiment with bacterial isolates from anoxic marine areas to determine average growth-limiting oxygen concentrations of about 1–4 μM, at the limits of then-current sensors. Many biogeochemical models prescribe a critical oxygen concentration in this range or higher, setting the transition to nitrate reduction and denitrification (e.g., Anderson et al. 2007; Najjar et al. 2007; Deutsch et al.

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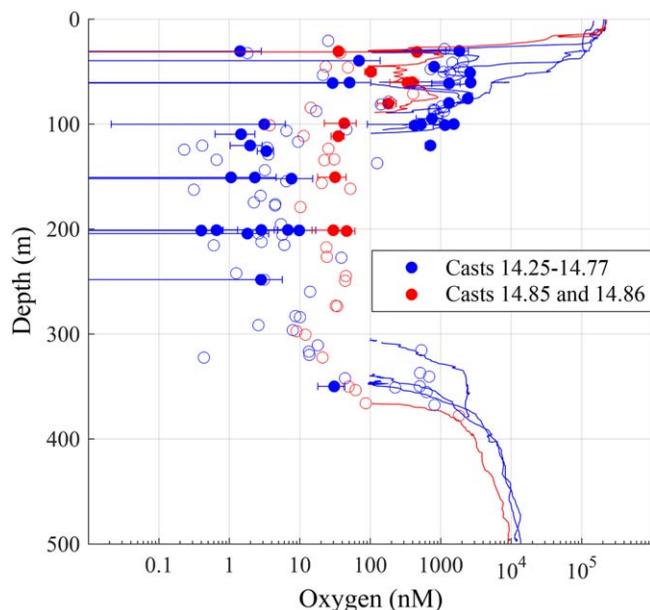
Fig. 1. Oxygen measured by Thamdrup et al. (2012) with the Switchable Trace amount OXygen (STOX) sensor (circles) and a STOX-calibrated conventional oxygen sensor (lines) in the eastern South Pacific ocean along the west coast of South America. As in Thamdrup et al. (2012), locations with multiple measurements (solid circles) include error bars (standard deviations), and locations with one measurement (open circles) have a detection limit of 10 nM. Error bars that intersect with the *y*-axis signify error to or below zero. Oxygen was consistently below 10 nM at the core of the anoxic zone at five sites, with nine total casts (blue), and varied from 10 nM to 50 nM at one site, with two casts (red).

2011; Bianchi et al. 2012; Gnanadesikan et al. 2012; Suntharalingam et al. 2012; Gutknecht et al. 2013). Brewer et al. (2014) proposed a theoretical basis for a critical oxygen concentration of this magnitude by considering the energy available from external oxygen and nitrate. Their analysis shows a higher energetic yield from the use of nitrate as an electron acceptor once oxygen drops to about 10 μM, with nitrate concentration at 40 μM. They interpret this as an upper-bound oxygen concentration for the onset of anaerobic nitrate reduction, pertinent when respiration is limited by the supply of an electron acceptor.

The recent development of the Switchable Trace amount OXygen (STOX) sensor has lowered the detection limit for dissolved oxygen measurements in the ocean from about 1 μM to 1–10 nM, and its deployment has revealed that large volumes of OMZ water have oxygen concentrations at or below these limits (Fig. 1; Revsbech et al. 2009; Jensen et al. 2011; Kalvelage et al. 2011; Thamdrup et al. 2012; Tiano et al. 2014). Although recent studies show the bulk of anaerobic activity occurring at the STOX detection limit, they also reveal denitrification and anammox at much higher concentrations (up to 10s of μM) both sporadically (Dalsgaard et al. 2012; De Brabandere et al. 2014) and consistently (Kalvelage et al. 2011). It is not clear whether these latter observations

can be explained by favorable anaerobic metabolisms at these concentrations, by micro-anoxic zones within particles (Karl et al. 1984; Woebken et al. 2007; Kalvelage et al. 2015; Klawonn et al. 2015), by experimental effects (De Brabandere et al. 2012), or by dispersal processes.

The STOX-enabled observations imply feasible aerobic growth at nanomolar levels of oxygen. Stolper et al. (2010) demonstrated aerobic growth of *E. coli* down to 3 nM oxygen in the laboratory, with data fit to a Monod model of oxygen-dependent growth with a half-saturation (K_m) value of 120 ± 20 nM. Complementary studies of OMZ microbes have used the STOX sensor and careful development of anoxic conditions to provide evidence of a nanomolar threshold between aerobic and anaerobic metabolism. Dalsgaard et al. (2014) demonstrated that oxygen suppresses denitrification rates, with 50% inhibition at about 200 nM and 300 nM for N_2 and N_2O production, respectively. Complementarily, Tiano et al. (2014) found that aerobic respiration continues until oxygen is depleted to nanomolar levels, with apparent K_m values of 10–200 nM. Gong et al. (2016) also measured varying K_m values of 30–60 nM for marine bacteria, some of which decreased to below 10 nM with changes in cell physiology. Dalsgaard et al. (2014) observed a 50% inhibition of anammox at much higher concentrations- almost 900 nM oxygen. Other observations also suggest that anammox tolerates higher oxygen concentrations than heterotrophic denitrification (Jensen et al. 2008; Kalvelage et al. 2011; Dalsgaard et al. 2012).

The physiological basis for these very low limiting oxygen concentrations is linked to underlying enzymatic affinities (Gong et al. 2016). Using spectrophotometric methods to indirectly measure oxygen, high-affinity terminal oxidases for oxygen have been identified with K_m values of 3–8 nM, which have been found to yield less energy per oxygen molecule than the low-affinity oxidases with K_m values around 200 nM (Bott and Niebisch 2003; Morris and Schmidt 2013). Most anaerobes are thought to be facultatively so, switching between oxygen and other terminal electron acceptors such as nitrate or nitrite (Zumft 1997), and may or may not encode the high-affinity terminal oxidases (Morris and Schmidt 2013). Those that do not may switch their cellular machinery away from aerobic growth at higher oxygen concentrations. Yet oxygen depletion to nanomolar detectability limits is widespread in marine oxygen minimum zones (e.g., Revsbech et al. 2009; Jensen et al. 2011; Kalvelage et al. 2011; Thamdrup et al. 2012; Tiano et al. 2014). Metagenomic analysis shows that the high-affinity oxidase is widespread in nature (Morris and Schmidt 2013), with both metagenomic and metatranscriptomic analysis showing its significance in the ETSP (Kalvelage et al. 2013, 2015). Hence the use of oxygen even at these very low levels must be a viable strategy in many environments, including OMZs.

Why is the minimum of dissolved oxygen in the ocean at or below nanomolar concentrations? In this study, we

present a theory for a dynamic oxygen limit for aerobic microbial growth. Assuming that aerobic prokaryotes control the minimum oxygen concentrations in the dark pelagic water column, we model a generic aerobic prokaryotic cell, and relate the uptake of oxygen to its physiological demand. The framework is sufficiently general to reflect chemoautotrophic, heterotrophic, and facultatively anaerobic metabolisms. We employ resource competition theory to frame the critical oxygen concentration as the minimum necessary to sustain an aerobic microbial population in a given environment, and suggest that the transition to energetically favorable anaerobic growth begins at this subsistence concentration, O_2^*. It follows that the ambient oxygen concentration is then maintained at O_2^* as anaerobic activity becomes significant, if no other sinks for oxygen are present. O_2^* is not a fixed concentration, but varies as a function of predatory and other loss rates, cell size, temperature, and the yield of biomass synthesis with respect to oxygen. The quantitative model shows that a wide range of conditions correspond to a nanomolar minimum oxygen concentration, with tenths to hundreds of nanomolar also plausible.

Derivation of O_2^*

We first consider how the supply of oxygen limits the growth of the generic aerobic functional type. We can calculate an oxygen-limited growth rate μ_{O_2}(time^{-1}) by relating the uptake rate of oxygen into a cell, ρ_{O_2} (mol O$_2$ cell^{-1} time^{-1}), to the yield of biomass with respect to oxygen, γ_{O_2} (mol C synthesized / mol O$_2$), and an estimate of the carbon quota of the cell, Q (mol C cell^{-1}), as:

$$\mu_{O_2} = \rho_{O_2} \gamma_{O_2} Q^{-1} \qquad (1)$$

The oxygen yield γ_{O_2} represents the moles of biomass synthesized per mole of oxygen respired for an aerobic heterotroph or chemoautotroph. Supporting Information Fig. 1(A) shows estimates of $\gamma_{O_2}^{-1}$ as the oxygen demand (mol O$_2$ / mol C synthesized), calculated as the ratio of bacterial respiration to bacterial production from the global database of community and bacterial respiration (version: 22 Jan 2015; Robinson and Williams 2005), which assumes a respiratory quotient of one (1 mol O$_2$ consumed = 1 mol CO$_2$ produced). The median oxygen demand is 5.4 mol O$_2$ / mol C (mean 11 ± 16 mol O$_2$ / mol C). The oxygen yield and organic matter yield (often referred to as the growth efficiency) of a heterotroph can also be related theoretically, based on mass and electron balance. We describe this prognostic approach in Supporting Information Appendix A.

Michaelis–Menten kinetics are used to describe the uptake of substrates, with the form dictated by a combination of factors, including diffusion through a molecular boundary layer, the density of porters, and/or the characteristics of the internal enzymes which utilize the substrate (Armstrong 2008; Fiksen et al. 2013). In Supporting Information

Appendix B, we show that at low oxygen concentrations and with high-affinity capabilities, the Michaelis–Menten model reduces to a linear, diffusive parameterization of transport across the molecular boundary layer (Gerard 1931; Gong et al. 2016), as:

$$\rho_{O_2} = 4\pi r D O_2 \qquad (2)$$

where r is the cell radius, D is the temperature-dependent diffusion coefficient for oxygen in seawater, and O$_2$ is the external concentration of oxygen. We consider this diffusive limit an appropriate description of a mixed microbial community, independent of K_m values. (Supporting Information Fig. 2; see Supporting Information Appendix B for discussion).

Resource competition theory then provides an ecological context. In a steady-state environment, a population has grown sufficiently to reduce a limiting resource, R, to its subsistence concentration, R^* (Tilman 1982). We evaluate the subsistence concentration of oxygen of the aerobic microbial functional type as the balance of oxygen-limited growth and loss rates. The rate of change of the biomass, B, neglecting physical transport and mixing terms, varies as a function of its growth μ and losses L, as:
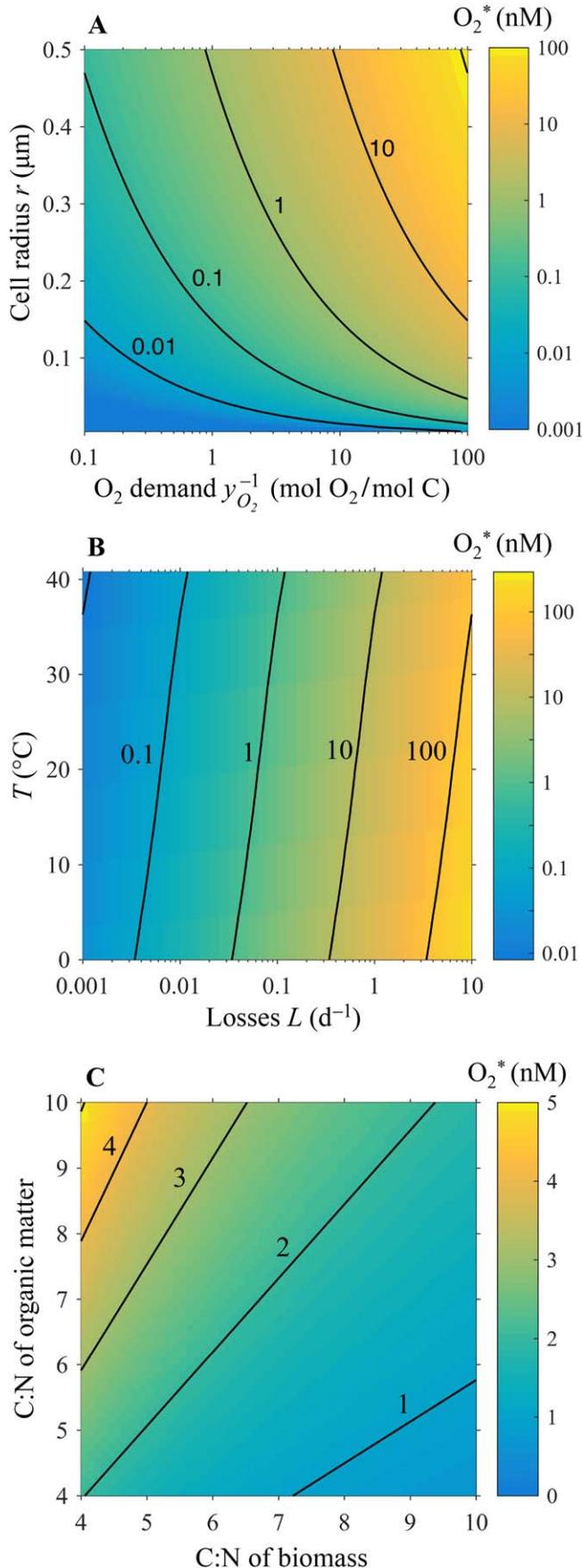
$$\frac{dB}{dt} = \mu B - L B \qquad (3)$$

where L represents all forms of loss and mortality, including maintenance metabolism (Pirt 1965), grazing, viral lysis and programmed cell death.

We make a steady-state assumption $\left(\frac{dB}{dt} = 0\right)$, which is approximately true for conditions in which $\mu B \approx L B$, and $\frac{dB}{dt} \ll \mu B$. Then combining Eqs. 1, 2, and 3 and including a more explicit description of the cell carbon quota ($Q = q\frac{4}{3}\pi r^3$, where q is a given volumetric carbon content of the cell; 18.3 fmol C μm^{-3} (Bratbak and Dundas 1984)) gives an expression for the subsistence oxygen concentration O_2^*:

$$O_2^* = \frac{L q r^2}{3 \gamma_{O_2} D} \qquad (4)$$

which is also the steady-state environmental concentration, given no other sinks of oxygen. This expression is general, and is relevant for an oxygen-limited microbial community with high-affinity capabilities (Supporting Information Appendix B). O_2^* is thus the concentration governing the viability of the aerobic metabolism at the population level.

Following resource ratio theory (Tilman 1982), once oxygen is depleted to O_2^*, coexistence of aerobic and anaerobic growth is feasible. We would not expect to observe energetically favorable anaerobic activity at oxygen concentrations higher than O_2^*, but aerobic and anaerobic metabolism can co-exist to varying degrees when oxygen is at this concentration. For a facultative anaerobic population, we can consider this variation as the fraction of the population's respiration

Fig. 2. O_2^* as a function of (**A**) cell radius r and oxygen demand $y_{O_2}^{-1}$, (**B**) losses L and temperature T (via D, the diffusion coefficient for oxygen in seawater), and (**C**) the C : N stoichiometry of organic matter substrate and biomass composition (Supporting Information Appendix A). Unless varying, parameters are set as best estimates for marine oxygen minimum zones: $L = 0.1$ d^{-1}, $T = 12°C$, $r = 0.25$ μm, and $y_{O_2}^{-1} = 7$ mol O_2 / mol C.

as a whole that utilizes oxygen vs. alternative electron acceptors.

Estimating O_2^*

Parameter space for O_2^* across marine environments

The subsistence concentration O_2^* for an aerobic microbe is not a constant; it is a function of the parameters in Eq. 4: losses L, cell size (here assuming a spherical cell with radius r), the oxygen yield y_{O_2}, and temperature (via the diffusion coefficient D for oxygen in solution). What is the plausible range of O_2^* in marine environments? Syntheses of observations that characterize most marine environments inform values for these parameters, and we illustrate the resulting range of reasonable O_2^* values in Fig. 2.

O_2^* increases linearly with oxygen demand $y_{O_2}^{-1}$. In Fig. 2A, we illustrate O_2^* over the range of $y_{O_2}^{-1}$ of 0–100 mol O_2 / mol C synthesized, representing a range in growth efficiency of 1–0.01 mol C synthesized / mol C consumed, respectively, assuming average marine stoichiometries (Anderson 1995; Supporting Information Appendix A). Mean bacterial growth efficiencies from the database of Robinson (2008) for open ocean and coastal regions (0.14 and 0.19, respectively) correspond to oxygen demands of 5–14 mol O_2 / mol C synthesized (Supporting Information Appendix A), although none of the data come from oxygen minimum zones. We note that O_2^* has an asymptotic relationship with growth efficiency (Eq. 8), since oxygen demand converges to zero as cell growth approaches perfectly efficient synthesis from organic substrate.

In Fig. 2A, we also illustrate O_2^* over the range in the size of marine heterotrophic bacteria: 0.15–0.5 μm in radius (Sherr and Sherr 2000). O_2^* increases quadratically with cell radius for a constant cellular carbon density q (18.3 fmol C μm^{-3}; Bratbak and Dundas 1984). Seawater temperature ranges from below 0°C in the deep ocean to over 30°C at the surface; we illustrate O_2^* over a range in temperature T of 0–40°C, which coincides with about a threefold variation in D of 1.1–3.6 x10^{-5} cm^2 s^{-1} for a salinity of 35 (http://www.unisense.com/files/PDF/Diverse/Seawater & Gases table.pdf, accessed 07 Jan 2016). O_2^* thus decreases slightly as temperature increases (Fig. 2B).

A relevant range for losses can be estimated from mean bacterial growth rates, since for a stable population at steady state, loss rates must balance growth rates. Bacterial growth rates at the surface vary substantially, but on average range

Fig. 3. Predicted O_2^* from measured vertical distributions of (**A**) temperature, oxygen, and (**B**) the specific grazing rate on bacteria by heterotrophic nanoflagellates at a coastal upwelling site off Iquique, Chile (20.06°S, 70.19°W; Cuevas and Morales 2006). O_2^* is calculated with and without an additional loss rate of 0.1 d^{-1}. For both estimates, (**C**) calculations reflect the average coastal growth efficiency of 0.19 (lines) with uncertainty due to its standard deviation of 0.16 (shaded regions). Measured oxygen concentrations (**A** and **C**) are omitted below the detection limit of 1 μM.

from about 0.1 to 1 d^{-1} (Ducklow 2000), with 0.1 d^{-1} as a calculated average (Kirchman 2016). Subsurface pelagic bacterial growth rates are also on average 0.1 d^{-1} (Aristegui et al. 2009). Since observations in OMZs are lacking, we illustrate O_2^* across a wider range of loss rates, from 10^{-3} to 10 d^{-1}, which represents population doubling times of 2 yr to 2 h, respectively (Fig. 2B). O_2^* increases linearly with loss rates.

Across the parameter space illustrated in Fig. 2, O_2^* varies from less than 0.1 to a few hundred nanomolar. Large values coincide with high oxygen demands, large cell sizes, and high loss rates. We next explore a more targeted parameter space.

Using observations to predict O_2^* in an OMZ

For predictions of O_2^*, total rates of losses—to grazing, viruses, maintenance, and cell death—are key (Fig. 2), yet poorly constrained for heterotrophic microbes in subsurface marine environments. Here, we use a dataset that specifically provides these loss rates for aerobic microbes to more carefully predict the minimum oxygen concentration in the core of an anoxic zone.

We calculate O_2^* from Eq. 4 for a site in the coastal upwelling region off of northern Chile using the observations of Cuevas and Morales (2006) of temperature and the specific grazing rates on bacteria by heterotrophic nanoflagellates (Fig. 3A,B). The diffusion coefficient for oxygen D was calculated as a function of temperature using a linear fit to published values for 2–25°C seawater (http://www.uni-sense.com/files/PDF/Diverse/Seawater & Gases table.pdf, accessed 07 Jan 2016; $R^2 = 0.998$). To increase the uncertainty of our estimate, we calculate O_2^* with and without the contribution of an additional 0.1 d^{-1} loss rate, representing

other mortality or maintenance. This gives two estimates of loss rates that differ by a factor of two (Fig. 3B). Cuevas and Morales (2006) infer that grazing rates fully compensate for bacterial production rates in the anoxic core below 40 m depth, which suggests that additional mortality is in fact negligible.

We then calculate O_2^* for $r = 0.25$ μm (which gives 14 fg C $cell^{-1}$ with the cellular carbon density (q) of Bratbak and Dundas (1984), close to the midpoint for the estimated 10–20 fg C $cell^{-1}$; Ducklow 2000) and using the coastal marine average growth efficiency and its standard deviation of 0.19 ± 0.16 (Robinson 2008) to estimate the oxygen demand (Supporting Information Appendix A). The model uncertainty (shaded areas) corresponds to the resulting range in oxygen demand of 3–35 mol O_2 / mol C. Because growth using the high-affinity oxidases, required to utilize oxygen at low levels, is presumably less efficient than that represented by the average, we may expect better predictive power of the higher end of this range (and higher resulting O_2^*).

The model predicts O_2^* consistently below 25 nM, decreasing slightly with depth (Fig. 3C). Although temperature and grazing rates both decrease with depth, their opposing influence (as illustrated in Fig. 2B) leads to a smaller net impact on O_2^*. In the "anoxic" core, below 40 m, the model predicts a mean O_2^* concentration of 0.5–3 nM, just at and below the STOX sensor detectability. The range in oxygen demand results in the model uncertainty of 0.2–24 nM, with the high end representing the highest oxygen demand (corresponding to a growth efficiency of 0.03 mol C synthesized / mol C consumed). This prediction could be partially tested, down to the nanomolar detection limit, by deployment of the STOX sensor at this location.

Beyond marine environments

Since low oxygen environments are not constrained to the marine realm, this model may have broader application. We compare marine estimates to the parameters documented by Stolper et al. (2010) for the growth of *E. coli* to 3 nM oxygen at 37°C, human body temperature. We find that the apparent half-saturation (K_m) values of bacteria in OMZs and *E. coli* in optimal laboratory conditions can be more than an order of magnitude different: < 10 nM and 120 nM, respectively (Stolper et al. 2010; Gong et al. 2016). Yet in both environments, oxygen is depleted to a few nanomolar or less (Stolper et al. 2010; Thamdrup et al. 2012). In Supporting Information Appendix B, we demonstrate how the diffusive supply of oxygen similarly limits prokaryotic growth in the deep ocean and in optimal laboratory conditions despite the different K_m values, since apparent K_m values reflect maximum rates (Fiksen et al. 2013). This suggests that this framework is sufficiently flexible to represent the limiting oxygen concentration for a variety of microbial populations growing at a variety of rates across environments.

Discussion

Summary of results

We hypothesize that the limiting oxygen concentration for aerobic respiration in an aquatic environment is O_2^*, the subsistence oxygen concentration for an aerobic prokaryotic population, and that O_2^* represents the minimum oxygen concentration in that environment. This anticipates the observed nanomolar oxygen concentrations in the ocean's oxygen minimum zones (e.g., Revsbech et al. 2009; Jensen et al. 2011; Kalvelage et al. 2011; Thamdrup et al. 2012; Tiano et al. 2014). As the threshold for sustainable aerobic growth, O_2^* is also the concentration at which external oxygen is maintained as diverse suites of anaerobic metabolisms activate, excepting the presence of other sinks for oxygen. This minimum oxygen concentration varies as a function of environmental factors as well as cell physiology: O_2^* increases with losses to mortality and predation, decreases with temperature, and increases with oxygen demand and cell size (Fig. 2). Plausible estimates for these factors in marine environments suggest that O_2^* may vary substantially—up to tens or hundreds of nanomolar for rapid microbial population turnover rates or high oxygen demand—but is largely in the nanomolar range. Using grazing rates on bacteria measured by Cuevas and Morales (2006) in a coastal anoxic zone, we predicted an O_2^* concentration of order 0.1–10 nM.

Implications of a flexible, nanomolar O_2^*
O_2^* as the minimum oxygen concentration in "anoxic" marine zones

We hypothesize that O_2^* is likely to represent the minimum ambient oxygen concentration of essentially anoxic pelagic marine zones. When $O_2 = O_2^*$, aerobic respiration is at the edge of being a non-viable metabolism for the microbe in its environment. Once oxygen is depleted to O_2^*, either coexistence with or competitive exclusion by an anaerobic metabolism is possible (Tilman 1982). The theory anticipates both in an anoxic zone: that aerobic respiration will maintain the O_2^* concentration while anaerobic metabolisms also operate, and that diverse anaerobic metabolisms may operate exclusively at the core of the zone.

In anoxic zones, $O_2 = O_2^*$ only if no other process is capable of further depleting oxygen. Sulfide oxidation in sulfidic environments could potentially scavenge oxygen to lower levels (Preisler et al. 2007; Canfield et al. 2010), thus excluding aerobic respiration entirely. Also, we have so far considered the O_2^* of strictly aerobic growth. If aerobic respiration and denitrification occur simultaneously in cells (Chen and Strous 2013), lowering a population's demand for oxygen relative to biomass synthesis, the O_2^* of that aerobic-anaerobic hybrid activity would be lower. Thus a hybrid metabolism would potentially further deplete oxygen as long as it remains energetically favorable. In these ways, the O_2^* of strictly aerobic growth represents an upper bound on the lowest oxygen concentration in an oxygen minimum zone.

O_2^* varies across environments

The O_2^* framework reflects variation among steady-state environments and provides an explanation for how the minimum oxygen concentration may differ among sampling sites and times and between different organisms adapted to various conditions. We might anticipate that the limiting oxygen concentration decreases with depth if bacterial grazing by nanoheteroflagellates, for example, decreases with depth. On the other hand, such an effect on O_2^* may be dampened or cancelled out by a decrease in growth efficiency with depth, since it is plausible that bacteria may optimize carbon utilization rather than their growth efficiency in the "oligotrophic" deep ocean (del Giorgio and Cole 1998).

At one site in the Eastern South Pacific OMZ, oxygen was consistently measured by the STOX sensor at 10–50 nM, in contrast to nine other casts at five sites, in which oxygen was below the detection limit (Fig. 1; Thamdrup et al. 2012). The authors point to a perturbation in the hydrography at this site as evidence of an injection of water from another source, probably by mixing. We may consider this higher oxygen concentration as indicative of aerobic activity. However, our analysis suggests that this 50 nM concentration may be the O_2^* concentration of the intruding water body, and thus it might also be undergoing anaerobic activity. Figure 2 shows that 50 nM is a plausible O_2^* concentration, reflecting, for example, a microbial population subject to a low growth efficiency and thus higher oxygen demand due to a less nutritious food source or some other energetic limitation. Simultaneous sampling for the presence of anaerobic activity could test this hypothesis. This case exemplifies how

the theory of a dynamic oxygen threshold can impact interpretation of observations: we do not expect one fixed limiting oxygen concentration for all environments.

An argument against a 10 μM threshold

Our results are quantitatively different from Brewer et al. (2014), who propose that nitrate should offer more free energy than oxygen once oxygen is depleted to about 10 μM, assuming nitrate concentrations of about 40 μM. They conclude that this could represent the conditions for the onset of nitrate reduction. Brewer et al. consider a case for which O_2^* theory does not apply: when growth is not limited by the electron donor (such as organic matter) but rather by the electron acceptor. This initially poses the question of whether or not these two theories are complementary, i.e., that a 10 μM onset for nitrate reduction and a nanomolar lower limit for aerobic respiration together represent a window for the energetically favorable coexistence of both.

Further analysis suggests not, with two lines of reasoning. First, the framework of Rittman and McCarty (2001), which serves as a base for O_2^* theory (Supporting Information Appendix A), poses that for this electron-acceptor-limited case the relevant comparative rates are the uptake rates of substrate into the cell relative to the yields of biomass for those substrates. These would be $\rho_{O_2} y_{O_2}$ for oxygen (as in Eq. 1) and $\rho_{NO_3^-} y_{NO_3^-}$ for nitrate. Brewer et al. consider diffusive supply rates for both oxygen and nitrate. But the fact that oxygen is a small, uncharged molecule that can passively diffusive into cell—in comparison to nitrate, which requires enzyme-controlled active transport—suggests that these two uptake rates differ significantly and consistently. In this way, the framework of Brewer et al. is relevant from a geochemical, but not microbial, perspective. From the microbial perspective, we consider O_2^* and a similarly-calculated NO_3^{-*} to be the comparable limits, and expect O_2^* to be consistently lower than NO_3^{-*} due to the diffusive-uptake advantage of oxygen.

Second, Brewer et al. consider the electron-acceptor-limited case, and we can further demonstrate that oxygen and nitrate concentrations of order 10 μM should not limit most marine microbial growth, and thus demonstrate that this case is rare. The model developed here quantitatively links external concentrations to growth. For oxygen to pose an energetic limitation to growth at 10 μM, growth rates would have to be at least about 50 d^{-1}, and over 100 d^{-1} for average efficiencies (Supporting Information Fig. 2). This could limit the > 140 d^{-1} growth rate of the fastest-growing marine heterotrophic bacteria, *Vibrio natriegens* (Maida et al. 2013; Kirchman 2016). But for most populations, organic matter processing or other internal constraints results in much lower rates (about 1 d^{-1} maximum, 0.1 d^{-1} on average; Kirchman 2016). We conclude that a 10 μM oxygen threshold can only be reconciled for the very fastest heterotrophic bacteria.

Potential for a higher O_2^* for chemoautotrophic metabolisms

We might assume that aerobic heterotrophs, due to a lower respiratory requirement per unit biomass, can draw down oxygen to a lower concentration than aerobic chemoautotrophs, such as nitrifiers, that undergo energy-intensive carbon fixation. This would imply that the switch from aerobic to anaerobic chemoautotrophy occurs at a higher oxygen concentration than the switch (within facultative cells) from aerobic to anaerobic heterotrophy. For example, we can consider the competition for ammonium between chemoautotrophic aerobic and anaerobic ammonia oxidation (i.e., the first step of nitrification and anammox), with the former using oxygen and the latter using nitrite as a terminal electron acceptor. All else being the same, the difference in O_2^* between nitrification and heterotrophy scales linearly with any difference in their oxygen demand (Eq. 4): if the nitrifying population requires 10 times more oxygen than the heterotrophic population to sustain the same rate of biomass turnover, its O_2^* will be ten times higher than that of heterotrophy. If this is 50 nM instead of 5 nM, for example, we might expect to see anammox occurring once oxygen is depleted to 50 nM, as it begins to favorably coexist with (and potentially eventually outcompete) nitrification at this higher O_2^*, even as oxygen continues to be depleted to 5 nM by heterotrophs. This is consistent with observations that anammox occurs at higher levels of oxygen than does heterotrophic denitrification (Jensen et al. 2008; Kalvelage et al. 2011; Dalsgaard et al. 2012, 2014).

However, Füssel et al. (2012) and Kalvelage et al. (2013) observe aerobic nitrification throughout oxygen minimum zones, suggesting that the O_2^* of nitrifiers may be comparable to that of heterotrophs. The smaller cell size of ammonia-oxidizing archaea (Martens-Habbena et al. 2009) or lower predation rates could allow for a comparable or even lower O_2^*. If this is the case, limitation by ammonium or nitrite, rather than oxygen, may govern chemoautotrophic dynamics.

Broad application

The theory here applies to oxygen minimum zones as well as to *E. coli* in the laboratory (Stolper et al. 2010): a simple, mechanistic model links oxygen-limited microbial growth to nanomolar oxygen concentrations (Supporting Information Appendix B). While Stolper et al. (2010) similarly conclude that the limiting oxygen concentration should increase with cell size, as postulated by Fenchel and Finlay (1995), we suggest that other factors are also important. The consistency of the model for two very different environments demonstrates a predictable limitation for aerobic microbial growth in diverse environments, and a broadly applicable model.

Limitations of O_2^* theory
The steady-state assumption vs. a dynamic oxycline

The results here define O_2^* for a steady-state microbial population, which is a valid approximation when the rate of change of the biomass of the population is small relative to its activity. The assumption applies to environments in which microbial metabolisms operate on much shorter time-scales than physical changes in the environment and thus control nutrient distributions. Departure from this steady state—perhaps from a pulse of quickly sinking organic matter—frees the threshold from the definition of O_2^*. In this way, O_2^* best describes the core of the anoxic zone and does not necessarily describe the diverse transition zone of a dynamic oxycline (Ward et al. 2008; Zaikova et al. 2010; Bryant et al. 2012).

Additionally, when local dispersal rates exceed microbial growth and loss rates, anaerobic cells may be swept away from their ideal O_2^* conditions but still carry out denitrification or other anaerobic metabolisms while adjusting their cellular machinery to their new surroundings. Depending on these adjustment timescales, such dispersal may allow for the documentation of "immigrant" anaerobic activity at higher concentrations (Clayton et al. 2013). Anoxic micro-environments inside particles (Karl et al. 1984; Woebken et al. 2007; Kalvelage et al. 2015; Klawonn et al. 2015) or methodological difficulties (De Brabandere et al. 2012) may also explain observations of anaerobic activity at tens of micromolar oxygen concentrations (e.g., Kalvelage et al. 2011; Dalsgaard et al. 2012; De Brabandere et al. 2014).

Other impacts on growth efficiency

If the efficiency of aerobic growth decreases as oxygen decreases, O_2^* will increase as oxygen decreases. Alternatively, if a facultative cell can acquire energy using oxygen and a form of nitrogen simultaneously, then O_2^* could decrease with decreasing oxygen, as nitrogen assumes a portion of the respiratory requirement. The model here is sufficiently general to incorporate either or both of these effects: as written, the model considers the oxygen yield as an independent variable. One could instead consider it as a dependent variable ($y_{O_2} = y_{O_2}(O_2)$), if this relationship is known.

However, if y_{O_2} decreases to the point that anaerobic metabolism becomes more efficient than aerobic, O_2^* theory no longer applies. For example, if the reduced efficiency of the high-affinity terminal oxidase system for oxygen utilization translates into a lower growth rate than that enabled by nitrate or nitrite utilization, the latter will be a more competitive strategy. Evaluating this difference in growth rate (i.e., how varying amounts of translocated protons of different oxidases relate to the competitive ability of aerobic and anaerobic cells at low oxygen concentration) would provide crucial insight, given that denitrification is much less efficient than its redox potential would suggest, for both bioenergetic as well as other kinetic reasons (Chen and Strous 2013). The fact that the STOX sensor has revealed large volumes of water at or below a few nanomolar concentrations suggests that utilization of oxygen to these low levels is a competitive strategy at those locations.

Utility of O_2^* theory for future observational and modeling work

The STOX sensor technology has already demonstrated nanomolar levels of oxygen in OMZs, and its attainable 1–2 nM detection limit could distinguish among oxygen concentrations within much of the predicted range for various environments. In this way, a sampling strategy could aim to analyze whether or not the minimum oxygen concentration actually does vary with the physiological and environmental parameters as predicted by the theory developed here. For example, concurrent measurements of temperature, bacterial production, bacterial respiration, and grazing rates on heterotrophic prokaryotes would enable a quantitative prediction of O_2^* (Eq. 4) that the STOX sensor could then test. Conversely, the precision of STOX measurements could be used in combination with a subset of these measurements to infer one of the physiological or environmental parameters, such as total loss rates for anoxic bacterial populations.

We understand O_2^* as the concentration at which energetically favorable anaerobic activity begins. Including the aerobic microbial functional type in a biogeochemical model would allow for the depletion of oxygen to nanomolar concentrations without prescribing a critical oxygen concentration. Including nitrification, anammox, and intermediate steps of heterotrophic denitrification as additional functional types would further predict rates of fixed nitrogen loss and other nitrogen cycle dynamics. Our approach thus points to a means of dynamically modeling the feedbacks between diverse microbial metabolisms and nutrient distributions in anoxic zones in global biogeochemical models.

Conclusions

We presented a theory for the depletion of oxygen to nanomolar concentrations in marine oxygen minimum zones. We hypothesize that the minimum oxygen concentration in many aquatic environments is the subsistence concentration, O_2^*, of the bulk aerobic microbial population. For environments under steady microbial control, we expect anaerobic metabolisms to be energetically favorable while this minimum concentration is maintained. The resulting model predicts that this threshold concentration varies with loss rates, cell size, growth efficiency, and temperature, and that the parameters describing marine environments constrain it largely to the 0.1–10 nanomolar range. The theory presented supports the understanding that the smallest microbes tolerate the lowest oxygen concentrations and thus inhabit low oxygen environments. The model also leads to a hypothesis for why anammox may be favorable at a higher

oxygen concentration than denitrification, which is implied by some observations. Consistency with the growth of *E. coli* in optimal laboratory conditions suggests that the framework and its nanomolar predictions apply broadly, spanning diverse microbes and environments. The model thus predicts the essentially anoxic oxygen concentrations observed in OMZs, reconciling theory with observations, and provides testable hypotheses for future field work. In general, the description of the aerobic microbial metabolism exemplifies a simple, mechanistic parameterization of the interactions between microbial communities and nutrient distributions suitable for global marine biogeochemical modeling, absolving the need for a prescribed critical oxygen concentration.

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Conflict of Interest

None declared.