1   # Biochemical Barriers on the Path to Ocean Anoxia?

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14   **ABSTRACT (154/250 words)**

15   The kinetics of microbial respiration suggest that, if excess organic matter is present, oxygen

16   should fall to nanomolar levels, in the range of the Michaelis-Menten constants (K_m). Yet even in

17   many biologically productive coastal regions, lowest observed O_2 concentrations often remain

18   several orders of magnitude higher than respiratory K_m values. We propose the *Hypoxic Barrier*

19   *Hypothesis* (HBH) to explain this apparent discrepancy. The HBH postulates that oxidative

20   enzymes involved in organic matter catabolism are kinetically limited by O_2 at concentrations far

21   higher than the thresholds for respiration. We found support for the HBH in a meta-analysis of

22   1137 O_2 K_m values reported in the literature: the median value for terminal respiratory oxidases

23   was 350 nM, but for other oxidase types the median value was 67 µM. The HBH directs our

24   attention to the kinetic properties of an important class of oxygen-dependent reactions that could

25   help explain the trajectories of ocean ecosystems experiencing O_2 stress.

26   **IMPORTANCE (87/150 words)**

27   Declining ocean oxygen associated with global warming and climate change is impacting marine

28   ecosystems across scales from microscopic planktonic communities to global fisheries. We

29   report a fundamental dichotomy in the affinity of enzymes for oxygen. The importance of this

30   observation has yet to be fully assessed, but it is predicted to impact the rate at which organic

31   matter is oxidized in hypoxic ecosystems, and the types of organic matter that accumulate.

32   Competition between intracellular enzymes for oxygen may also have impacted microbial

33   strategies of adaptation to suboxia.

34   **KEYWORDS**

35   oxygen minimum zones, oxygenase K_m, ocean respiration, dissolved organic matter

36   **INTRODUCTION**

37   Marine suboxic and anoxic zones are hotspots of microbially-mediated biogeochemical

38   transformations that regulate the nitrogen budget and air-sea fluxes of greenhouse gases of the

39   global ocean (1). Because dissolved oxygen (DO) also organizes the structure and dynamics of

40   ocean food webs, understanding the processes that regulate expansion of suboxic and anoxic

41   zones in response to past and current climate changes is a pressing challenge (2). Suboxic and

42   anoxic zones are embedded within broader oxygen minimum zones (OMZ) that comprise some

43   8% of the surface area of the ocean. While recent advances in nanomolar-scale DO measurement

44   technologies have enabled precise delineation of the presence of suboxia and anoxia (3), we

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45 contend that a perplexing yet fundamental question has been overlooked. Given our canonical

46 understanding of microbial respiration kinetics, why are suboxia and anoxia not a much more

47 pervasive feature of the ocean's low oxygen zones?

48 Of biological reactions that consume O_2, by far the most important, in terms of mass, is carbon

49 respiration. Michaelis-Menten half saturation (K_m) constants for respiration are typically very

50 low, on the order of a few nanomolar, although higher values have been reported (4) (**Figure 1**).

51 Thus, if labile organic carbon, i.e. compounds that readily can be used as a source of electrons

52 for respiration, is delivered in excess to a microbial ecosystem, DO declines at a rate determined

53 by the respiratory capacity of the microorganisms present and the supply of organic matter.

54 Importantly, the minimum DO attainable should reflect the well-described high-affinity,

55 nanomolar scale K_m of microbial respiratory oxidases (5). Other factors that can influence DO in

56 aquatic ecosystems include: photosynthesis, when light is present; oxygen transport by ocean

57 currents and mixing; diffusion, which can limit respiration, particularly in aggregates of cells;

58 impacts of low oxygen on grazing metazoa (6), which require higher oxygen concentrations than

59 bacteria; non-respiratory biochemical reactions that consume oxygen; and abiotic reactions that

60 consume oxygen (7). Nonetheless, DOM formation and oxidation is the mechanistic centerpiece

61 in our fundamental understanding of microbial-scale processes leading to low oxygen states and

62 predictions of global ocean oxygen dynamics.

63 Eastern boundary upwelling systems (EBUS) represents one of the ocean's most productive

64 biomes. In these coastal ecosystems, oxygen-poor subsurface waters uplifted from the vertical

65 periphery or core of open ocean OMZs receive elevated organic carbon inputs from surface

66 phytoplankton blooms. **Figure 2** shows relative water volumes for DO concentrations across

67 these systems. Of the ocean's four major EBUS, only the Peru-Chile Current System in the

68   Eastern Tropical Pacific Ocean persistently exhibits DO-deficient states in continental shelf

69   waters. For both Pacific EBUS, there is an accumulation of water volumes below 100 µM but a

70   sharp drop-off in volume of waters that reach suboxic (<5 µM) or anoxic (~ 0 µM) states in the

71   upper ocean (0-400 m) including continental shelf waters where remineralization and oxygen

72   loss is most active (Fig 2b). The pattern is striking - despite the nanomolar-scale of respiratory

73   K_m values, respiration in productive EBUS is able to draw down DO to hypoxic levels but rarely

74   is able to consume the last 10-60 µM DO. While the depth of OMZs extends below 400 m, the

75   failure of suboxic and anoxic volumes to accumulate despite the presence of large volumes of

76   hypoxic water persists when we expand our sampling to 1000 m (Fig 2d).

77   To explain the observations in **Figure 2**, we propose the ***Hypoxic Barrier Hypothesis (HBH),***

78   **which states:** ***dissolved O_2 kinetically limits the activity of oxygenase enzymes involved in the***

79   ***breakdown of organic matter, in the range of oxygenase K_m values (median value 67 µM)***

80   ***causing a decline in DOM oxidation rates in ecosystems experiencing oxygen stress, and an***

81   ***accumulation of DOM that is catabolized by pathways that require oxygenases.*** The *HBH*

82   ascribes the decline in O_2 frequency distributions of suboxic and anoxic waters (Figures 2 and

83   S2) to fundamental biochemical properties of cells, particularly the mechanisms by which

84   oxidative enzymes cleave semi-labile organic matter, making it accessible to further oxidation.

85   **Oxygen depletion by respiration in aquatic systems.** How far can respiring marine bacteria

86   lower oxygen concentrations when they are provided with an ample supply of reductant for

87   respiration, as would be expected for plankton in the presence of an excess of labile organic

88   carbon? The Pasteur Point is an influential concept based on the observation that facultative

89   anaerobes switch to fermentation at ca. 2.2 µM O_2, approximately an order of magnitude below

90   the average K_m for oxygenases (Fig 1), steep declines in suboxic and anoxic water volumes in

4

91   EBUS (Fig 2), and the ca. 25 µM inflection in cumulative frequency distribution of DO

92   observations recorded in CCS (Fig. S2). Newer information suggests that the limits of bacterial

93   respiration are in the nanomolar range. This is consistent with the observation of high affinity

94   cytochromes that exhibit O_2 K_m between 3 and 200 nM (8; Figure 1). Higher O_2 Michaelis

95   constants have been sometimes been reported for marine bacteria, but it has been suggested that

96   higher values obtained with whole cells reflect diffusion limitation, which can be expected to

97   inflate apparent O_2 Michaelis constants in proportion to cell sizes and respiration rates. Stolper et

98   al. showed that *E. coli* cells could grow at less than 3 nM O_2, a sufficiently low concentration to

99   limit growth by diffusion, but high enough to sustain growth through O_2 respiration (8). Our

100   meta-analysis of indicates that cells grown on highly labile carbon compounds such as glucose

101   display whole cell O_2 K_m values extends well into nanomolar O_2 concentrations. We conclude

102   that a substantial background of observations and theory support the conclusion that the

103   respiration rate of chemoheterotrophic cells should not by limited by O_2 at concentrations found

104   in ocean hypoxic zones or the Pasteur Point at ca. 2.2 µM.

105   The accumulation of hypoxic and scarcity of suboxic or anoxic volumes in the ocean nonetheless

106   suggest that negative feedbacks between oxygen decline and respiration may be at play. Direct

107   measurements of microbial O_2 K_m in natural systems are rare but available evidence point to K_m

108   values far higher than the nanomolar values reported from laboratory cultures with labile carbon

109   sources. Working in the Arabian Sea OMZ, Keil et al. (9) observed an apparent K_m of 20 µM O_2

110   for microbial community respiration. In the Nambian and Peruvian OMZ, Kalvelage et al. (10),

111   reported a linear decline in respiration rate between 20 and 0 µM O_2. In Chesapeake Bay, a

112   hypoxia-prone system, microbial respiration rates saturate at $[O_2]$ above 25 µM (11), a pattern

113   that we have similarly found for the CCS OMZ (Figure S3). Holtappels et al. (12) further

114   reported linear declines in respiration rates between 14 and 1 μM O_2 in waters collected from a

115   fjord in Denmark. These results are surprising because researchers using the same methods have

116   also found many instances of nM K_m values for microbial respiration. This suggests a bimodal

117   distribution of O_2 K_m values that differ by upwards of three orders of magnitude. Telescoping out

118   further, global models of ocean O_2 and carbon export converge on K_m values of between 4 and

119   20 μM O_2 in order to optimize fit between model and observations (13, 14). What accounts for

120   the disparity between accumulation of hypoxic water volumes, the μM scale K_m's reported from

121   natural systems and used to fit models, and nM scale K_m's predicted by respiratory oxidases?

122   Biochemistry offers a mechanistic explanation for this apparent disparity. There is evidence in

123   the scientific literature suggesting that microbial respiration of some types of organic matter is

124   slows when oxygen concentrations fall low enough to inhibit catabolic oxygenase enzymes.

125   Kroonman et al. (15), studying 3-chlorobenzoate degradation by the bacterium *Alcaligenes*,

126   reported two K_m values for O_2 uptake. They attributed the lower value (65 nM) to respiration

127   and the higher value (7-17 μM) to the activity of dioxygenases. Leahy and Olsen (16), studying

128   toluene degradation by *Pseudomonads*, also reported biphasic kinetics for toluene catabolism as

129   a function of oxygen concentration. The slope of the oxygen response declined with an inflection

130   at 20 - 30 μM O_2. In both of these cases the behavior of the cultured cells oxidizing recalcitrant

131   compounds is remarkably similar to the generalized behavior of ocean ecosystems approaching

132   hypoxia.

133   To further explore the distribution of O_2 K_m values among biological reactions, we conducted a

134   metanalysis of published data, shown in **Figure 1; Figure S1A; Table S1**. For O_2 K_m values

135   reported in the literature, the median value for terminal respiratory oxidases was 350 nM, but for

136   other oxidase types the median value was 67 μM. The difference of ~100 fold in median values

137   was supported by a p-value of <2e-16 in a t-test of the linear mixed-effect model coefficient

138   comparing the log-transformed O_2 K_m values (17). The bimodal distribution of O_2 K_m observed

139   at the enzyme scale is also repeated in whole cell studies. Cells that are grown on more complex

140   organic carbon sources have a median respiratory K_m value of 20 μM, while cells grown on

141   highly labile organic carbon such as glucose have median K_m of 690 nM (Figure S1B).

142   Many enzymes that catalyze the biological breakdown of organic matter use oxygen as a

143   substrate, yielding partially oxidized products that are metabolized further through catabolic

144   pathways. These enzymes are often classified as either monooxygenases (mixed function

145   oxidases) or dioxygenases. Enzymes in both families evolved to use O_2 as a substrate, but

146   monooxygenases incorporate a single oxygen atom into the substrate, reducing the second atom

147   to water, whereas dioxygenases typically add both atoms of the reacting O_2 to the product. A

148   geochemically important example of a monooxygenase is the heme-dependent Mn peroxidase

149   that catalyzes oxidation of lignin, a phenolic oligomer. Fungal ligninases belong in the heme-

150   dependent peroxidase superfamily (18). Ligninases evolved in the Paleozoic, and it has been

151   postulated that their origin resulted in widespread biodegradation of wood, causing the end of the

152   carboniferous period, and rises in global atmospheric CO_2 (19). Another superfamily of oxidases,

153   the flavin-dependent monooxygenases, are among the most diverse and prevalent proteins known.

154   They catalyze a wide range of reactions, for example hydroxylation, Baeyer–Villiger oxidation,

155   oxidative decarboxylation, epoxidation, desulfurization, sulfoxidation and oxidative denitration

156   (20). Many of the reactions catalyzed by flavin-dependent monooxygenases initiate the

157   catabolism of compounds that are otherwise recalcitrant to oxidation. These enzymes share a

158   common mechanism in which reduced flavin reacts with oxygen to produce a flavin C4a-

159   (hydro)peroxide that then reacts with electrophilic or nucleophilic substrates, typically resulting

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160   in the consumption of one diatomic oxygen molecule, the addition of an oxygen atom to the

161   substrate, and the release of water. Also important are the dioxygenases, which belong to a

162   different protein family also feature prominently in DOM degradation particularly for aromatic

163   compounds. These protein families came to the attention of oceanographers recently when it was

164   discovered that cells of one of the important oceanic bacterial clades, SAR202, harbor expanded

165   clusters of paralogous genes from both of these protein types (21). It has been proposed that

166   these enzymes participate in the oxidation of semi-labile organic matter, initiating its breakdown.

167   The meta-analysis of $O_2 K_m$ values presented in Figure 1 suggests that micromolar DO sensitivity

168   is ubiquitous across metabolic processes. Among the "other oxidase" types, we observed no clear

169   trends that associated $O_2 K_m$ values with protein families sorted by COGs or other precise

170   functional groups, such as enzyme commission classifications. In contrast, cytochrome

171   respiratory proteins with heme cofactors consistently displayed a much higher affinity for

172   oxygen that other protein types. Phylogenetically, the distribution of $O_2 K_m$ values included

173   diverse bacteria, including Proteobacteria, Actinobacteria, Firmicutes, and Cyanobacteria, as

174   well as eukaryotic organisms including fungi, humans, and other chordates. $O_2 K_m$ values showed

175   a 100-fold difference between respiratory and non-respiratory oxidases regardless of taxonomic

176   group. We conclude that this pattern is robust to phylogenetic bias in $O_2 K_m$ value sampling.

177   While our focus in the current work is marine systems, the HBH in principle applies to all

178   ecosystems.

179   **The role of oxidases in organic matter degradation.** Oceanographers classify organic matter

180   by its half-life, frequently using the category "labile dissolved organic matter" (LDOM) to

181   describe dissolved organic matter that is oxidized in minutes to hours, or at most a few days,

182   while the term "semi-labile" (SLDOM), and sometime "recalcitrant" are used to refer to

183   dissolved organic matter that persists longer, but is eventually oxidized. Here we introduce a new

184   term, "oxygen-dependent DOM" (ODDOM) to describe DOM that is catabolized via reactions

185   that require the activity of oxygenases and thus are susceptible to inhibition when O_2

186   concentrations reach values in the range of ca. 10-100 μM. We'll confine the discussion to

187   dissolved forms of organic matter, although most organic matter enters ecosystems as particulate

188   organic matter (POM) and is subsequently converted to DOM before being used by

189   microorganisms. Implicit in the above categories is the idea that different kinds of organic

190   matter are accessible to biological oxidation through different mechanisms and at different rates.

191   The HBH is consistent with the distribution of ocean anoxic zones if one assumes organic

192   matters supplies are uneven. If LDOM is oversupplied relative to oxygen, for example by high

193   rates of export production in systems with restricted circulation, then the activities of respiratory

194   terminal cytochrome complexes would be expected to readily draw down DO to nM

195   concentrations in accordance with their nM K_m values. In natural systems, LDOM are rapidly

196   depleted. As hypothesized, the activities of non-respiratory oxidases limit the supply of reductant

197   to respiratory oxidases. This acts as a bottleneck that slows the rate of respiration as DO declines.

198   With sufficient time, DO should reach minimum values as expected from nM K_m values of

199   respiratory oxidases. Such conditions can be met in the core of OMZs that have been isolated

200   from the atmosphere over decadal to century time scales, and evidence of this can be seen in Fig.

201   2.

202   The large disparity in K_m's we report between respiratory oxygenases and other oxygenase types

203   has implications for microbial cell evolution and metabolic regulation at the cellular level.

204   Inside of cells respiratory oxygenases could outcompete other oxygenases, exacerbating the

205   slowing of some oxygen-dependent cellular processes at low oxygen. To avoid this, cells may

206   have evolved metabolic regulation that avoids such competitive interactions, for example by

207   shifting to alternate electron acceptors before O_2 is depleted (22). This topic, which needs

208   exploration, could help us understand how microbial cells have adapted to suboxic environments,

209   which are far more common in the ocean than anoxic environments.

210   **Testing the HBH.** The HBH sets forth a number of central predictions that are testable by

211   experimentation, observation, and modeling. The impact of biphasic oxygen dependence

212   predicted by the HBH should be manifested as a broad potential for oxygen to limit microbial

213   respiration across hypoxic systems in the range of oxygenase K_m values (median value 67 µM),

214   when LDOM is depleted, but not if excess LDOM is present. To test that prediction, we

215   measured rates of respiration (oxygen uptake) in water samples from the Northern California

216   Current System OMZ, where DO minimum reach only ~5 µM, well above canonical nM K_m for

217   cytochrome oxidases. DO was increased by the simple expedient of allowing air to be

218   momentarily entrained during filling (**Figure S3**). In our experiments, and other similar

219   experiments we found among published work, the addition of DO caused respiration rates to rise

220   relative to controls. This observation could be attributed to the limitation of respiration by

221   diffusion (20), but alternatively, it could result from mechanisms described in the HBH model

222   we propose.

223   There are many other experimental avenues to testing the HBH that have not been explored.

224   **Figure S2** scratches the surface of what could done with field experiments and mesocosms to

225   verify predictions of the HBH. For example, experiments that test the biological availability of

226   DOM at high (e.g. 200 µM) and moderate (e.g. 20 µM) DO could challenge these ideas.

227   Mechanisms invoked by the HBH would lead to changes in the chemical composition of DOM

228   as DO declines: the ratio of LDOM to ODDOM should decrease as DO approaches the K_m

229   values of catabolic oxygenase enzymes for O_2. Measurements of DOM chemistry could

230   determine whether these changes occur as predicted. ODDOM, a term coined herein to segregate

231   DOM into categories by chemical composition and oxygenase involvement in catabolism, is at

232   present a theoretical concept, albeit grounded in the fundamentals of biochemistry. Although

233   chemical oceanographers do not at present measure ODDOM, in principle methods such as high

234   resolution NMR, HPLC, and LC-MS/MS could be applied for this purpose, and could be used to

235   test predictions of the HBH. Omics approaches, including functional genomics, provide an

236   avenue that could be applied in marine systems to measure the expression and activity of

237   oxygenase enzymes involved in ODDOM metabolism, and to characterize of the responses of

238   plankton cells and communities to suboxia.

239   The HBH has broader implications that could be explored with global data. It posits that rates of

240   oxygen loss and DOM oxidation slow as DO approaches hypoxia, setting the upper bounds for

241   the size of oceanic anoxic zones and organic carbon pools within. This can be evaluated in detail

242   by modeling studies that test the sensitivity of model-data comparisons to changes in

243   assumptions about microbial kinetic constants for oxygen.

244   **Alternatives to the HBH.** While we propose HBH to explain declines in oxygen frequency

245   distributions at unexpected high values (Figs. 2 and S2) and the rarity of suboxia and anoxia

246   across productive, low oxygen EBUS, alternate scenarios could explain this phenomenon. For

247   example, consistent barriers to oxygen diffusion to the terminal oxidases of respiratory systems,

248   or feedback mechanisms involving the production of sulfides and/or depletion of DO in

249   microhabitats, or oxygen limitation of metazoan grazing, could play a role in suppressing

250   respiration at low oxygen concentrations. Alternatively, there may be constraints on supply of

11

251   organic carbon or positive feedbacks on the resupply of DO by advection or diffusion as DO

252   approaches hypoxia.

253   **Public interest and policy.** The relevance of this issue to public interests in ecosystem

254   management could not be more profound. Ocean deoxygenation, the decline in ocean oxygen

255   inventories, has emerged as a leading pathway for climate change impacts in the sea. This

256   decline has been linked with expansion of hypoxic and anoxic zones. Oxygen deficient zones are

257   hotspots of biogeochemical transformations whose growth can have profound impacts on marine

258   biodiversity, vertical organic carbon flux, the sustainability of fisheries and feedbacks that

259   govern ocean nitrogen budgets and flux of radiatively active N_2O. The ability to accurately

260   forecast such ecosystem changes is central for informing responsive climate change mitigation

261   and adaptation policies. However, the disagreement between observations and the textbook

262   understanding of microbial respiration raises fundamental questions about the mechanisms that

263   underlie our conceptual and numerical models of the ocean dynamics as climate change

264   intensifies. The HBH offers a testable framework for examining a potentially flawed

265   fundamental principle that governs our thinking about OMZ formation. If this hypothesis is

266   correct, it will open previously overlooked avenues of research at the intersection of oxygenase

267   enzyme evolution, oxygenase-dependent metabolism in microbial communities, and OMZ

268   dynamics.

269   **Conclusion.** If these ideas have the power to even partially explain the kinetics of ocean oxygen

270   depletion, they could contribute to a better understanding of climate change impacts on ocean

271   deoxygenation and DOM chemistry. The data in Fig. 1 show us that the HBH is founded on

272   sound basic principles, but the impact of oxygenase "barrier" we describe is relative to many

273   other processes, mentioned above, that can also slow respiration, most notably diffusion. Sorting

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274 out the magnitude of catabolic oxygenase enzyme contributions to DOM oxidation, whether that

275 number be large or small, will help us assess how the trajectories of aquatic systems

276 experiencing oxygen declines are shaped by the fundamental biochemistry described in the HBH.

277 **MATERIAL AND METHODS**

278 **Data collection.** Scientific literature was mined for characterized oxygenase enzymes with

279 published K_m values for dissolved oxygen for both individual enzyme assays as well as whole

280 cell assays (Table S1). Metadata, including enzyme name and host scientific organism name,

281 were extracted from each article. We used a combination of BRENDA enzyme database, uniprot

282 protein database, and KEGG database searches to determine putative protein accessions, KEGG

283 ortholog IDs, and EC numbers associated with the published enzyme data. Repeated entries for

284 the same organism-protein pairs were included due to the various testing conditions per study.

285 Basin and global inventories of DO volumes were compiled from the World Ocean Atlas 2018

286 (https://www.ncei.noaa.gov/products/world-ocean-atlas. Dissolved oxygen observations (5-

287 400m) from CTD profiles were compiled from Chan et al. 2008, https://www3.mbari.org/bog/,

288 and https://www.calcofi.org/ for the northern (n=107,032 1950 to 2006), central (n=4,372 1997-

289 2013), and southern (n=4,372 1997-2013) CCS, respectively.

290 **Respiration rate experiment**. Water samples were drawn from above and within the CCS OMZ

291 (46 47.56°N, 125 11.83°W, 1000m station depth) and filled into 300ml borosilicate glass BOD

292 bottles that each contained an oxygen optode dot (PreSens Precision Sensing GmbH). On filling,

293 DO in a subset of samples initial [DO] were increased by allowing air to be entrained momen-

294 tarily in the Niskin outflow tubing. Bottles were incubated in a ~6°C water bath in the dark. DO

295 change over 48 was measured through the glass via detection of phase shift luminescence.

296

297 **Boxplot generation.** Km-DO values were split into two primary groups dependent upon general

298 protein function, either respiratory oxidases or non-respiratory oxidases. K_m-DO values were

299 also gathered for whole cells, whereby labile and semi-label carbon sources were compared,

300 mirroring the respiratory and non-respiratory individual enzyme assays. Plots were generated

301 with the grouped Km-DO values using R v4.0.2 (23) and the ggplot2 (24). A linear mixed

302 effects model was used to control for repeated O_2 Km measurements from the same organism,

303 with the formula $\log(K_m) \sim$ oxidase type + (1 | organism). The model was fit using maximum

304 likelihood, and the t-test to confirm significant difference of the coefficient for oxidase type was

305 done using Satterthwaite's method for degrees of freedom (17). Log (natural) transformed values

306 were used to approximate normality in the data. R code for this analysis can be found at github

307 repo: https://github.com/davised/HBH-2021.
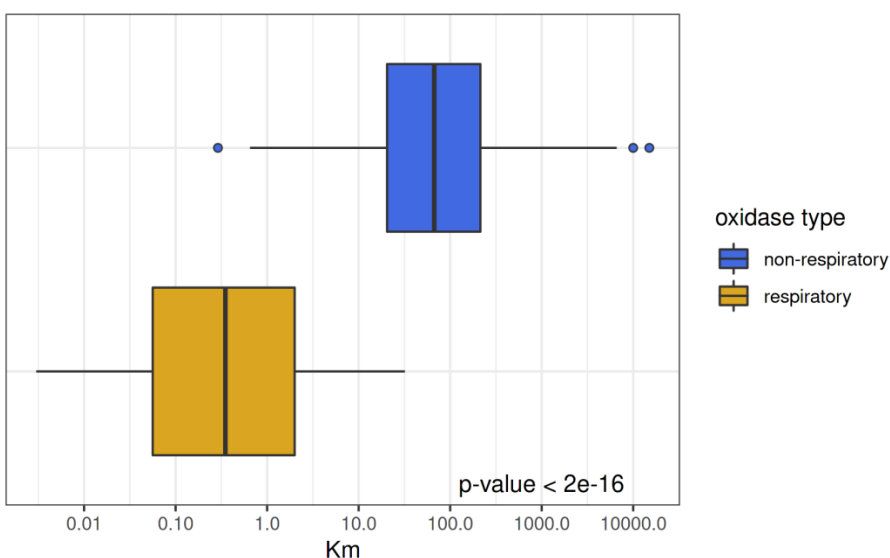
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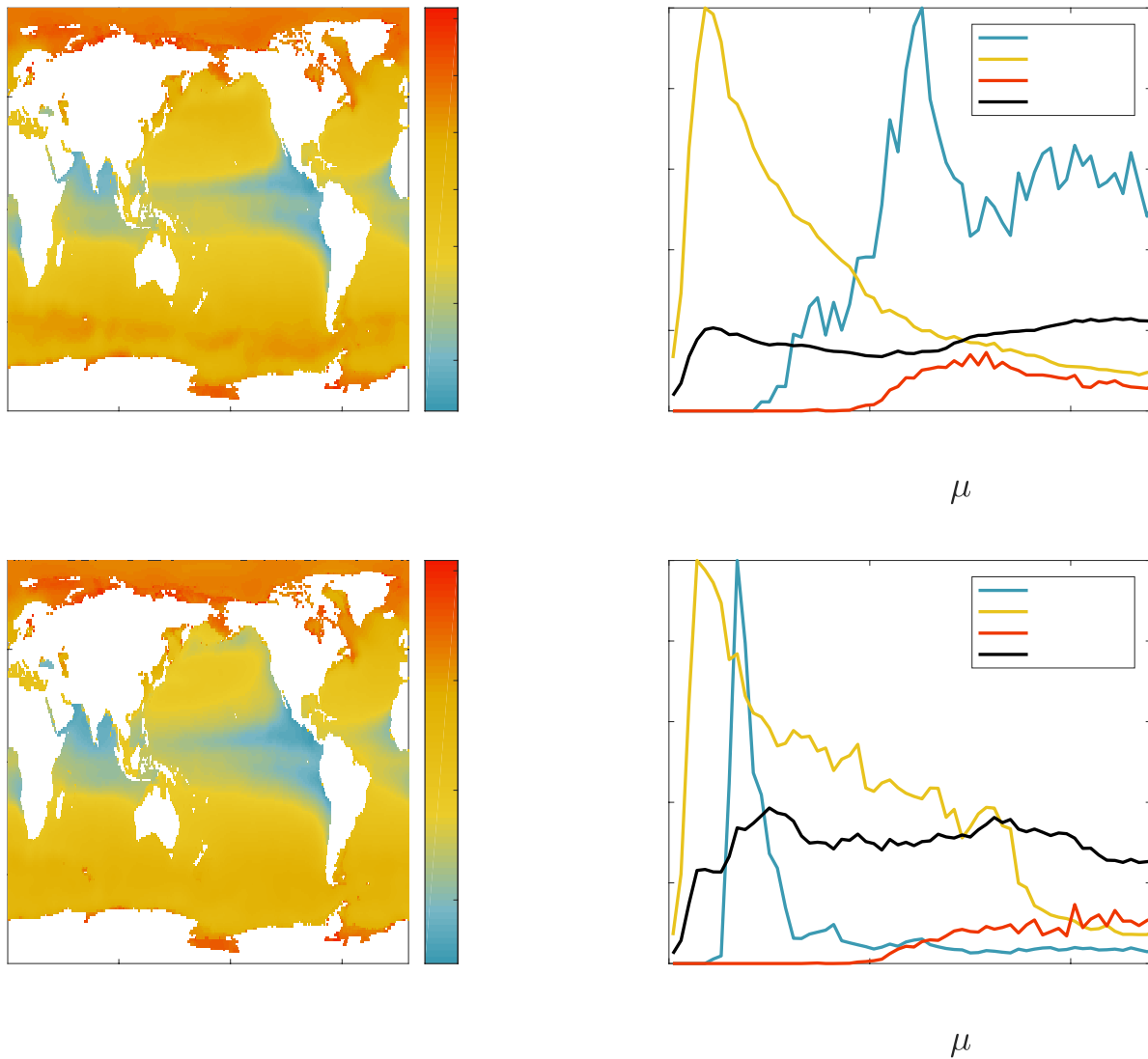
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382

383   **FIGURES**

384

385   **Figure 1.** K_m values are significantly smaller in respiratory oxidases compared to other

386   oxygenases. K_m-DO (dissolved oxygen) values for respiratory oxidases (yellow; n=109) and

387   other oxygenases (blue; n=890) are depicted on a log10 scale. The relatively high (e.g. 10^1 μM)

388   K_m values reported for oxidase enzymes indicate a potential bottleneck in the supply of electrons

389   from organic matter to respiration. The reported p-value is from a t-test using the Satterthwaite

390   approximations to degrees of freedom of a linear mixed model fit by maximum likelihood. Data

391   and citations can be found in Table S1.

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Figure 2. Climatological DO concentrations and distribution of ocean volume across DO

concentrations relationships for the California Current System (CCS), Eastern Tropical Pacific

(EastTropPac), Eastern Tropical Atlantic (EastTropAtl) and global ocean. (A) 0-400 m,

represents generally annual to decadal-scale processes, relative to ventilation and biomass

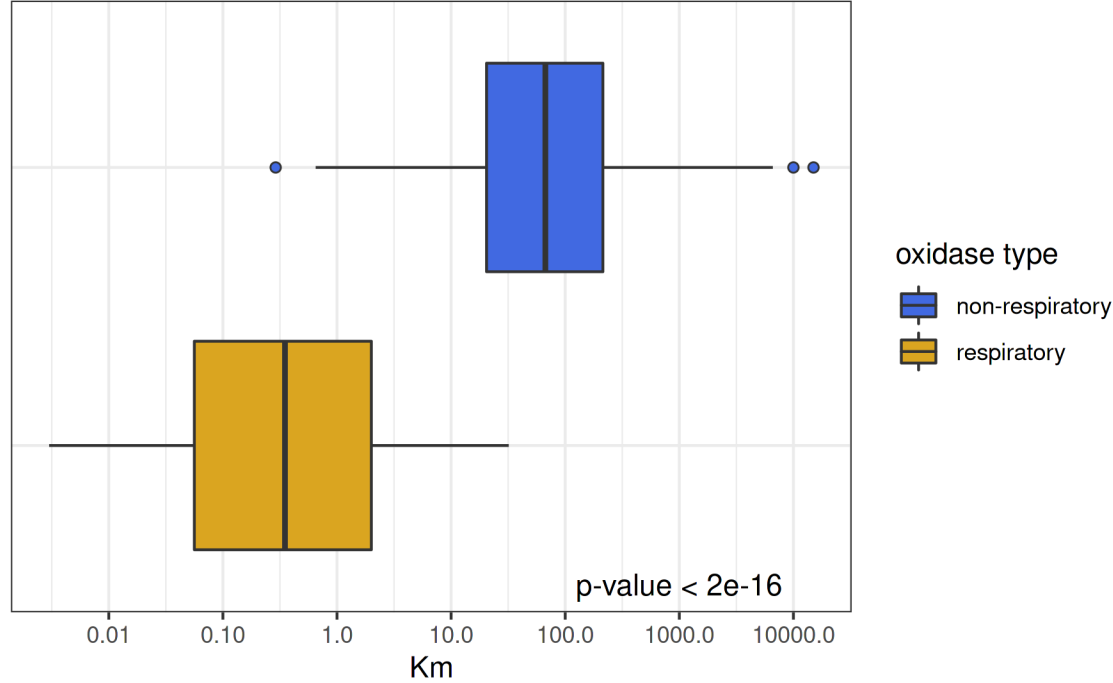formation by photosynthesis, (B) 0-1000 m, encompasses decadal to centennial-scale processes.

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400 **SUPPLEMENTAL MATERIALS**

401 **Table S1.** Literature reports of K_m values for oxygenase enzymes and terminal respiratory

402 oxidases used to construct Figs 1 and S1.
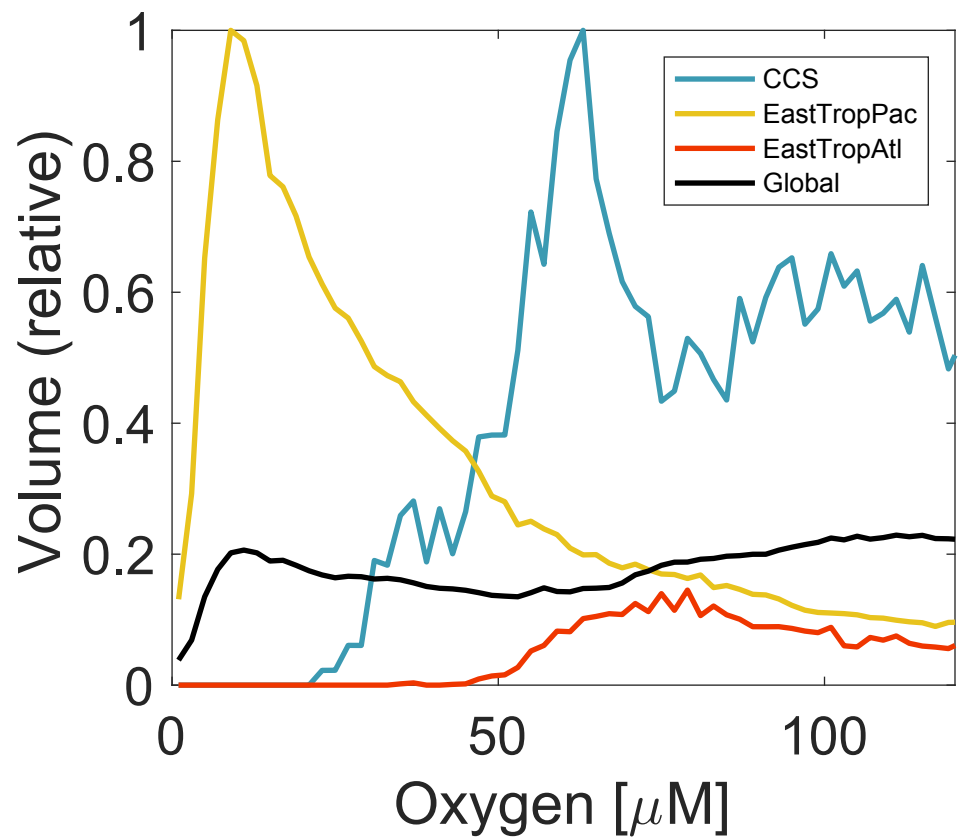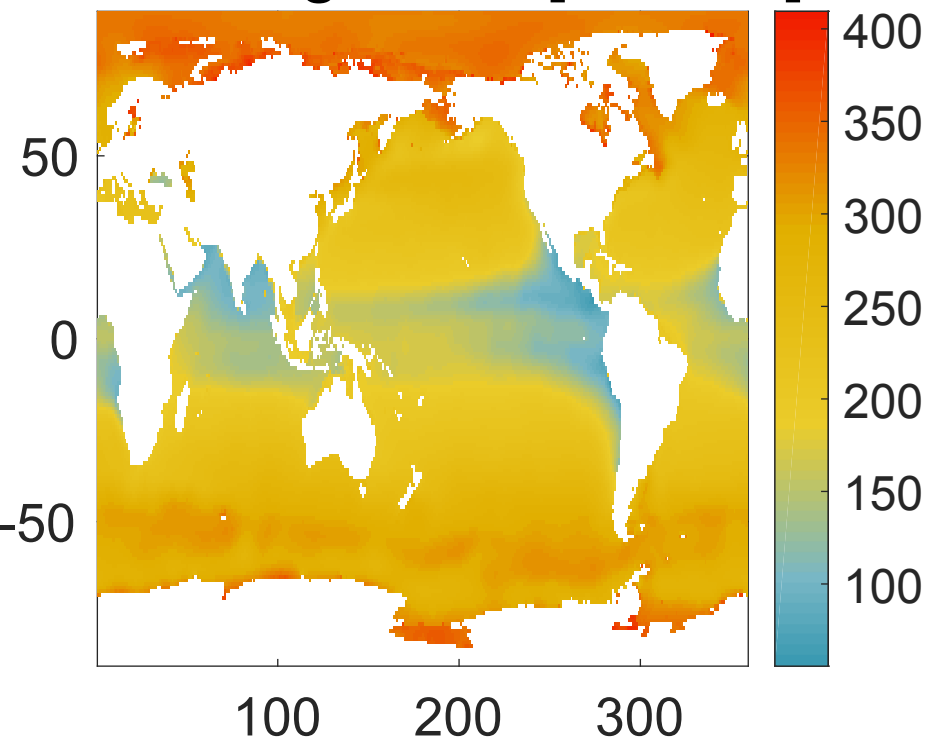
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404 **Fig. S1.** Oxidase density plot. This plot illustrates the distribution of oxygenase K_m values

405 reported in the literature for respiratory and non-respiratory oxidases (A) compared to whole cell

406 assays including labile and semi-labile carbon sources (B).

407

408 **Fig. S2.** Cumulative frequency distribution of DO observations from continental shelf depths (5-

409 400 m, i.e. above the OMZ) for the northern (—), central (—), and southern (—) California

410 Current System (CCS). Note the rarity of DO observations < 25 μM and an apparent hinge in the

411 frequency of observations as DO increases beyond that concentration.

412 **Fig. S3.** Examples of respiration rates from field collected samples that exhibited non-saturating

413 dynamics at 10's of uM [O_2]. In each instance, respiration rates were assayed in samples where

414 [O_2] was manipulated independently. Data from the CCS were measured via O_2 optode equipped

415 glass bottles from water samples collected within the OMZ. Oxygen was increased by allowing

416 air to be momentarily entrained as bottles were filled.

417

Climatological O2 [0-400 m]

Climatological O2 [0-1000 m]