

## 1 **Supplemental Methods**

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### 3 **Measurement of Biological O<sub>2</sub>**

4 O<sub>2</sub>/Ar was measured continuously during the cruise from the *R/V Atlantic Explorer's*  
5 underway intake using Equilibrator Inlet Mass Spectrometry (Cassar et al 2009). The ship's inlet  
6 is located approximately 2.5 meters below the waterline. The O<sub>2</sub>/Ar ion current ratio was  
7 recorded from the headspace of the membrane contactor, which undergoes gas exchange and  
8 equilibration with the sampled seawater, for 2-3 hours at a time, punctuated by 30-minute  
9 measurements of the O<sub>2</sub>/Ar of ambient room air to correct for instrumental drift. An automated  
10 Valco valve (VICI, Houston, TX, USA) governed the regular switching between air and water  
11 O<sub>2</sub>/Ar gas sample flow.

12 At each CTD station during the cruise, triplicate Winkler dissolved oxygen samples were  
13 collected from both in-situ surface water and from the ship's seawater tap directly upstream of  
14 the EIMS to measure the degree of microbial respiration within the ship's lines, a phenomenon  
15 that has been documented in the underway seawater systems of multiple research vessels  
16 (Juraneck et al 2010). All Winkler sample bottles were collected according to the standard BATS  
17 protocol and analyzed at Rod Johnson's laboratory at the Bermuda Institute of Ocean Sciences.

18 As in previous O<sub>2</sub>/Ar work conducted aboard the same vessel (Estapa et al 2015), O<sub>2</sub>  
19 measurements revealed insignificant differences between the dissolved oxygen concentration of  
20 surface seawater and seawater collected from the ship's underway seawater lines, suggesting that  
21 any microbial respiration taking place within the ship's underway system was negligible.

22

### 23 **Calculation of net community production**

24 Mass spectrometer and ship underway data were time-averaged at two-minute intervals.  
 25 Seawater O<sub>2</sub>/Ar values were calibrated using O<sub>2</sub>/Ar measurements of ambient room air, then  
 26 quality-filtered to remove O<sub>2</sub>/Ar data collected during anomalous spikes in mass spectrometer  
 27 pressure. Data was filtered according to a moving average and a moving standard deviation  
 28 criterion, removing data collected whenever pressure within a one-hour window exceeded a  
 29 moving standard deviation of 10<sup>-7</sup> mbar and/or a moving average greater than 5 x 10<sup>-6</sup> mbar.  
 30 Daily NCP rates were calculated using the following equation (Cassar et al 2011):

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$$32 \quad 1) \text{ NCP (mmol O}_2\text{/m}^3\text{/day)} = \frac{O_2(\%)}{Ar} * \frac{[O_2]_{sat}(\frac{umol}{kg})}{1000} * \rho(S, T) * k * (\frac{1}{MLD})$$

33

34 Where  $\frac{O_2}{Ar}$  (%) is the percent supersaturation of the O<sub>2</sub>/Ar ratio relative to that of interpolated  
 35 ambient air O<sub>2</sub>/Ar measurements, which reflect the expected O<sub>2</sub>/Ar ratio at equilibrium.  $\rho$   
 36 signifies the density of seawater (kg/m<sup>3</sup>) according to the 1983 UNESCO equation of state, and  $k$   
 37 denotes the piston velocity, estimated according to the quadratic wind-speed dependence of  
 38 (Wanninkhof 1992). MLD is the mixed layer depth in meters. The oxygen concentration  
 39 corresponding to saturation was calculated using the oxygen solubility relationship described in  
 40 (Garcia and Gordon 1992).

41 As biological oxygen concentrations in the surface ocean are also dependent on past wind  
 42 speeds, gas transfer velocities were weighted using the 30-day wind speed history prior to  
 43 measurement (Reuer et al 2007), with previous wind speeds along the cruise track obtained from  
 44 the corresponding daily NCEP/NCAR 10 m wind speed reanalysis. Mixed layer depths for this  
 45 calculation were calculated using potential density profiles obtained from fourteen CTD casts  
 46 according to a density difference criterion of 0.03 kg/m<sup>3</sup> from a reference depth of 10 m (de

47 Boyer Montegut et al 2004). Calculated mixed layer depths were linearly interpolated across the  
 48 cruise track between CTD casts.

49 As NCEP/NCAR wind speed values corresponding to cruise dates and locations appear to  
 50 underestimate ship-measured wind speeds, the ship-measured values were corrected to a 10 m  
 51 reference height using Equation (1) of (Thomas et al 2005) and used to adjust the NCEP/NCAR  
 52 wind speed history based on a linear regression between predicted and measured wind speeds for  
 53 the cruise period.

54

### 55 **Accounting for vertical fluxes of O<sub>2</sub>/Ar**

56 The integrated biological oxygen concentrations in the mixed layer can be expressed via  
 57 the following equation:

$$58 \quad 2) \quad MLD \frac{dO_{2_{bio}}}{dt} = NCP - F_g + F_v + F_e$$

59  $O_{2_{bio}}$  represents the concentration of biological oxygen in the surface ocean.  $F_g$  denotes the gas  
 60 exchange term, while  $F_v$  and  $F_e$  represent vertical fluxes via eddy diffusion and entrainment.

61 Traditionally, the system is assumed at steady-state with limited  $F_v + F_e$  (Hamme and Emerson  
 62 2006), with  $F_g$  equal to NCP, leaving just  $F_v + F_e$  to consider. We estimated the vertical eddy  
 63 diffusive flux  $F_v$ , using equation (6) of (Castro-Morales et al 2013), with a vertical eddy  
 64 diffusivity coefficient of  $1 \times 10^{-5} \text{ m}^2 \text{ s}^{-1}$  as measured for the Mid-Atlantic Bight (Wallace 1994),  
 65 calculating oxygen gradients from CTD profile data using a Model-II least-squares fit. Estimated  
 66 depth-integrated  $F_v$  fluxes ranged between just 0.2 and 5.35  $\text{mmol O}_2 \text{ m}^{-2} \text{ day}^{-1}$  (median: 1.41  
 67  $\text{mmol O}_2 \text{ m}^{-2} \text{ day}^{-1}$ ). These flux estimates likely represent an upper bound, as much of the  
 68 observed increase in  $O_2$  beneath the mixed layer may be thermally rather than biologically-  
 69 driven. Argon vertical profiles are however likely close to equilibrium within the first few

70 hundred meters, minimizing errors associated with using O<sub>2</sub> concentration profiles rather than  
71 O<sub>2</sub>/Ar (Hamme and Severinghaus 2007).

72         Entrainment due to deepening of the mixed layer is unlikely to be significant in the  
73 highly stratified summer Sargasso Sea. A similar study in the Sargasso Sea in September-  
74 October 2011/2012 assessed entrainment flux to comprise less than +/- 6% of calculated O<sub>2</sub>/Ar-  
75 derived NCP (Estapa et al 2015). We are, however, unable to account for entrainment fluxes in  
76 the more dynamic coastal region. O<sub>2</sub> concentrations below the mixed layer consistently increased  
77 with depth based on CTD profiles taken throughout our cruise, however, with the result that any  
78 entrainment fluxes would bias NCP values upwards. On the other hand, observed negative NCP  
79 values in open-ocean regions of the Sargasso Sea likely represent true heterotrophic activity.

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#### 81 **Determination of appropriate control DNA spike quantity**

82         We conducted a qPCR using 18S V4 primers to assess the average concentration of 18S  
83 rDNA genes in our samples. Serial dilutions (1:10 to 1:1 000 000) of the *S. pombe* control DNA  
84 were used to generate the qPCR standard curve. The sample with the lowest 18S DNA  
85 abundance was determined to contain approximately 1/4th the number of 18S rDNA sequences  
86 ul<sup>-1</sup> as 1.14 ng of the *S. pombe* standard (9 702 000 copies), suggesting an appropriate addition of  
87 0.00285 ng to maintain the standard sequences added at a relative abundance of no more than 1%  
88 of reads following sequencing.

89         We subsequently conducted an Ion Torrent PGM sequencing run to verify our ability to  
90 detect such a dilute spike in our sequencing output data. Two sets of samples from the 2015  
91 cruise were run in technical duplicates: one pair from an oligotrophic location near Bermuda and  
92 another from a large phytoplankton bloom south of Long Island. These sites were chosen to span

93 the range of 16S and 18S rDNA gene concentrations encountered in this study. These samples  
94 were extracted with the addition of 152 ng or 15.2 ng of 16S and 0.679 ng or 0.0679 ng of 18S  
95 internal standards, both in 50 ul volumes, following the same extraction and PCR protocols  
96 described in the main text. Ion Torrent PGM sequencing was performed at the Boston University  
97 Microarray Core on a 314 chip. Primer, barcode, and adapter sequences used for this Ion Torrent  
98 sequencing run are detailed in Supplementary Table 6.

99

## 100 **Statistical Analysis**

101 For comparison to sequenced samples, underway NCP data was time-averaged across 45-  
102 minute bins centered on the time of sample collection. If sample collection took place during a  
103 gap in NCP measurements, a 22.5-minute average of the closest NCP data to the sample location  
104 was used instead. If the time difference between sample collection and the nearest NCP data  
105 exceeded three hours, then the station was assigned the median value for NCP across the entire  
106 cruise track. The latter approach was only implemented for three sample stations (S06, S24, S25)  
107 [Supplementary Table 2]. Prior to reaching stations 24 and 25, technical issues prevented further  
108 O<sub>2</sub>/Ar data collection. The nearest NCP data to these stations differ from the cruise median (~0.2  
109 mmol O<sub>2</sub> m<sup>-3</sup> d<sup>-1</sup>) by less than 0.21 mmol O<sub>2</sub> m<sup>-3</sup> d<sup>-1</sup>. The closest NCP data to S06 is virtually  
110 identical to the cruise median rate (<.03 mmol O<sub>2</sub> m<sup>-3</sup> d<sup>-1</sup> difference). Given the low number of  
111 stations at which this approach was used, the location of these stations in the oligotrophic ocean,  
112 and the small differences between the estimated station rates and the nearest recorded data—all  
113 of which are in near-balance with the atmosphere in terms of biological O<sub>2</sub> saturation—any bias  
114 to the dataset from this approximation is likely negligible.

115 Statistical analyses were performed on 16S rDNA and 18S rDNA taxonomy data using  
116 the vegan (Okansen et al 2017) and phyloseq packages in R.

117 Ordination of samples was performed with Principal Coordinate Analysis (PCoA), using  
118 weighted Bray-Curtis dissimilarity to express ecological distance. The first principal component  
119 of each projection was further modeled against the dataset of NCP and environmental  
120 measurements using linear regression. For these and all subsequent statistical comparisons with  
121 our molecular data, which represent volumetric sequence counts, NCP rates were expressed as  
122 volumetric values ( $\text{mmol m}^{-3} \text{d}^{-1}$ ), though we obtained similar results if mixed-layer integrated  
123 NCP values were used.

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### 125 **Partial Least Squares Regression Analysis**

126 Partial least squares (PLS) regression analysis was performed using the ‘mixOmics’  
127 package in R (Rohart et al 2017) in ‘regression’ mode with two model components to identify  
128 bacterial and eukaryotic taxa with good predictive power in estimating NCP and environmental  
129 variables. Analyses were performed at the taxonomic levels corresponding to the order level for  
130 both the 18S and 16S datasets, excluding less-abundant taxa that comprised <0.5% of total 18S  
131 or 16S reads. Leave-one-out cross-validation was performed to evaluate model performance.

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### 133 **Online data availability**

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135 Sequences and metadata are available from the NCBI Sequence Read Archive under accession  
136 number SRP126177. Other data available upon request.

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