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Microbial respiration in the euphotic zone at Station ALOHA

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Abstract

Measurements of the temporal and vertical variability of microbial community respiration (MCR) in the euphotic zone (0–200 m) at Station ALOHA were made using the *in vivo* INT method to constrain oxygen and carbon cycling at this oceanic site. Mean ($\pm$ 1 SE) MCR was higher in the upper (0–100 m) (0.89 ± 0.05 mmol O_2 m^{-3} d^{-1}) than in the lower (100–200 m) portion of the euphotic zone (0.52 ± 0.05 mmol O_2 m^{-3} d^{-1}). Respiration in the 0.8 μm size-fraction relative to respiration in the 0.2–0.8 μm size-fraction was on average 1.4 ± 0.1. Variability in MCR was observed on both daily and monthly time scales, suggesting that respiration is a dynamic process throughout the year at Station ALOHA. MCR in the 0.2–0.8 μm size fraction was more variable than >0.8 μm MCR. Despite significant vertical and temporal variability in MCR, the euphotic zone depth-integrated (0–200 m) MCR was relatively constant (134.8 ± 11.8 mmol O_2 m^{-2} d^{-1}) throughout the period of observation. Oxygen consumption via MCR always exceeded O_2 production extrapolated from ^{14}C-primary production estimation, assuming a photosynthetic quotient of 1.13 mol O_2 produced : mol CO_2 fixed. MCR plus particulate carbon export from the euphotic zone for the period November 2011–October 2012 at Station ALOHA can be used to set a lower limit of $\sim$ 45 mol C m^{-2} yr^{-1} for gross primary production.

Marine microbes play an important role in global nutrient cycling (Arrigo 2005) and, in particular, the oceanic carbon cycle (Azam 1998). Marine phytoplankton are responsible for nearly half of Earth's primary production (Field et al. 1998), and mineralization of organic matter in the upper layer of the ocean is controlled by heterotrophic microbial communities. The balance between oxygen (O_2) production via gross primary production (GPP), and O_2 consumption via respiration (R) determines the net metabolic state of the ecosystem. If GPP > R in the euphotic zone (0–200 m), then excess organic matter and O_2 are available for export to other oceanic and atmospheric reservoirs. Hence, the assessment of microbial community respiration (MCR) is fundamental for a comprehensive understanding of the role of marine microorganisms in the global carbon and oxygen cycles (del Giorgio and Duarte 2002; del Giorgio and Williams 2005).

The paucity of MCR measurements is considered to be one of the main obstacles limiting our understanding of carbon flow in the open ocean (Williams and del Giorgio 2005; Morán et al. 2007; Gasol et al. 2008; Robinson 2008). Most field work has focused on photosynthesis (i.e., CO_2 reduction/O_2 production) rather than respiration in large part due to detection limits of methods used to measure changes in O_2 during timed incubations (Robinson and Williams 2005; Robinson 2008). Recently, a novel indirect method to estimate MCR (the *in vivo* INT method) has proven to be useful even in oligotrophic marine ecosystems (Martínez-García et al. 2009, 2013; Teira et al. 2010).

Herein, we report temporal and vertical variability of MCR (total, 0.2–0.8 μm and >0.8 μm particle size fractions) in the euphotic zone at Station ALOHA in the North Pacific Subtropical Gyre. We use these new data to set a lower constraint on GPP, the foundation of the microbial food web, to assess the microbial carbon cycle in this well-studied oligotrophic ecosystem.

Methods

Study site and sampling

Samples were collected at Station ALOHA (22°45′N 158°00′W) between November 2011 and October 2012 during Hawaii Ocean Time-series (HOT) cruises 236, 238–243 and 247, and Center for Microbial Oceanography: Research and Education (C-MORE) cruise Hawaii Ocean Experiment: DYnamics of Light And Nutrients 9 (HOE-DYLAN 9). Euphotic zone samples (0–200 m) were collected in 12-l PVC bottles attached to the CTD rosette sampling system. Environmental data for each experiment are presented in Table 1.

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Table 1. Initial conditions (mean and standard error) at Station ALOHA during the period of observation. Mixed layer depth, surface temperature, depth-integrated (0-200 m) primary production rates, particle flux at 150 m, depth-integrated (0-200 m) chlorophyll *a* concentration, depth-integrated (0-200 m) *Prochlorococcus* spp. abundance and depth-integrated (0-200 m) heterotrophic bacteria abundance are presented. Values in brackets are the Hawaii Ocean Time-series mean and standard error and the value range (http://hahana.soest.hawaii.edu/hot/hot-dogs) from 1989 to 2012 (2006-2012 in the case of flow cytometry samples).

	Cruise and Date								
Variable	HOT 236 11/4/2011	HOT238 12/19/2011	HOT 239 1/18/2012	HOT 240 3/25/2012	HOT 241 5/1/2012	HOT 242 5/30/2012	HOT 243 6/26/2012	HOE–Dylan9 8/26/2012	HOT 247 10/7/2012
Surface Temperature (°C)									
	25.9	23.9	23.6	23.0	23.5	24.3	24.4	25.6	25.6
	(25.5+0.1)	(24.7 ± 0.1)	(23.7 ± 0.1)	(23.5 ± 0.1)	(23.7 ± 0.1)	(24.4 ± 0.1)	(25.3 ± 0.1)	(26.0 ± 0.1)	(26.2 ± 0.1)
	(24.7–26.5)	(23.8–25.7)	(22.7–24.8)	(22.8–24.5)	(22.6–24.7)	(23.0–25.5)	(24.1–26.3)	(25.3–27.0)	(25.5–26.8)
Mixed layer depth (m)									
	57.1 ± 2.2	117.6 ± 2.8	98.1 ± 6.7	63.9 ± 4.8	114.1 ± 2.2	36.5 ± 1.5	73.0 ± 2.1	40.5 ± 2.2	59.9 ± 2.0
	(70.9 ± 4.3)	(83.5 ± 4.8)	(95.1 ± 5.7)	(55.4 ± 4.8)	(57.8 ± 7.2)	(39.8 ± 4.5)	(43.9 ± 2.4)	(48.6 ± 1.4)	(59.1 ± 2.7)
	(34.7–108.5)	(20.8–117.6)	(52.3–127.8)	(20.7–96.4)	(22.8–128.2)	(13.2–72.1)	(29.4–73.0)	(34.3–62.1)	(38.3–80.8)
Chlorophyll *a* (mg m^{-2})									
Σ0–200	30.3 ± 0.5	24.8 ± 0.2	30.2 ± 0.8	25.3 ± 0.0	29.9 ± 5.9	22.3 ± 7.3	22.3 ± 1.7	22.2 ± 0.0	21.8 ± 9.8
	(23.1 ± 0.9)	(23.4 ± 0.9)	(24.4 ± 1.2)	(23.4 ± 0.9)	(24.1 ± 1.0)	(24.4 ± 0.8)	(22.2 ± 1.1)	(24.3 ± 0.9)	(24.9 ± 1.5)
	(18.1–54.7)	(17.0–34.2)	(15.7–39.1)	(14.1–39.5)	(18.4–33.6)	(15.7–29.9)	(15.1–35.5)	(17.3–32.6)	(18.9–32.5)
Primary Production (^{14}C–PP) (mg C m^{-2} d^{-1})									
Σ0– 200	522.7 ± 7.4	n/a	528.3 ± 9.5	n/a	756.8 ± 20.1	616.6 ± 19.6	691.1 ± 17.8	617.8 ± 9.2	556.1 ± 34.6
	(443.2 ± 28.4)	(384.8 ± 22.5)	(416.9 ± 25.1)	(525.3 ± 25.9)	(537.6 ± 31.9)	(631.8 ± 26.2)	(611.2 ± 40.3)	(631.0 ± 27.8)	(495.8 ± 25.2)
	(286.5–704.2)	(173.0–491.9)	(286.5–778.2)	(394.2–865.9)	(233.6–807.1)	(423.2–927.1)	(297.4–1078.5)	(474.0–859.0)	(219.6–710.5)
Prochlorococcus spp. abundance (×10^{11} cells m^{-2})									
Σ0– 200	210.5	198.7	191.8		257.6	254.9	214.8	261.05	221.8
	(232.0 ± 15.1)	(199.8 ± 15.3)	(222.2 ± 20.2)	(220.1 ± 23.2)	(240.5 ± 12.9)	(243.9 ± 2 3.7)	(254.5 ± 29.8)	(239.2 ± 12.3)	(262.2 ± 11.9)
	(184.8–264.9)	(184.8–264.9)	(168.1–288.9)	(165.4–279.0)	(214.2–275.1)	(189.0–323.4)	(210.0–311.1)	(202.8–280.3)	(233.7–286.2)
Heterotrophic bacteria abundance (x10^{11} cells m^{-2})									
Σ0–200	758.7	780.0	718.6		850.7	741.9	729.8	835.32	788.2
	(789.4 ± 25.1)	(743.1 ± 37.9)	(674.7 ± 53.9)	(699.8 ± 52.5)	(774.3 ± 54.4)	(793.3 ± 25.3)	(796.8 ± 48.6)	(778.5 ± 52.4)	(836.4 ± 31.4)
	(638.0–885.2)	(675.5–793.8)	(595.1–883.4)	(607.2–850.6)	(641.1–891.5)	(627.8–894.8)	(699.8–850.3)	(557.7–894.5)	(788.3–925.1)
Particulate carbon flux (150 m) (mg C m^{-2} d^{-1})									
	31.9 ± 1.6	n/a	10.7 ± 1.3	22.6 ± 2.3	46.8 ± 2.3	45.0 ± 4.6	33.9 ± 2.2	22.0 ± 1.4	23.3 ± 1.0
	(23.6 ± 1.4)	(20.5 ± 1.7)	(21.9 ± 1.6)	(30.1 ± 1.9)	(29.2 ± 2.2)	(32.4 ± 2.2)	(32.8 ± 1.9)	(34.6 ± 2.5)	(25.9 ± 2.0)
	(16.2–31.9)	(13.8–44.0)	(10.7–33.4)	(17.9–47.5)	(17.3–49.3)	(17.7–56.1)	(20.0–49.5)	(19.0–51.6)	(10.7–57.0)

In vivo INT reduction rates

The reduction of the tetrazolium salt 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride (INT) to INT-formazan (INT-F) by Electron Transport System (ETS) dehydrogenase enzymes was used to estimate MCR. Size-fractionated respiration rates were routinely obtained using the *in vivo* INT method (Martínez-García et al. 2009).

Briefly, samples (two killed controls and three live, 250 mL each) were spiked with a sterile solution of INT (8 mM final concentration) and incubated in the dark for 1.5 h before dawn (i.e., the same time that incubation for ^{14}C-based primary production began) in shipboard incubation chambers. Formaldehyde-killed controls (2% v/v final concentration) were performed to account for any abiotic reduction of INT (Martínez-García et al. 2009), however formaldehyde-sensitive abiotic reduction (i.e., possibly associated with cell free enzymes) is not accounted for in the killed controls. Initial time-course experiments (incubation times up to 24 h were tested) were performed to determine the optimal incubation time, and the single 1.5 h procedure was used throughout the study. Incubation temperature was maintained ± 1°C of the *in situ* temperature. After incubation, samples were fixed by adding formaldehyde (2% v/v final concentration) and sequentially filtered through 0.8 μm and 0.2 μm pore size polycarbonate filters. Respiration in the size-fraction >0.8 μm is referred to as >0.8 μm MCR and respiration in the size-fraction 0.2–0.8 μm is referred to as 0.2–0.8 μm MCR. Total MCR is reported as the sum of respiration in both size-fractions (i.e., >0.8 μm MCR + 0.2–0.8 μm MCR). The respiration in the >0.8 μm size-fraction relative to respiration in the 0.2–0.8 μm size-fraction (i.e., >0.8 μm MCR/0.2–0.8 μm MCR) is referred to as 0.8/0.2 μm MCR. The reduced INT-F was extracted from the filters using propanol and its concentration determined by absorption spectrophotometry (Ultra Violet-2401 PC Shimadzu spectrophotometer). To transform ETS activity into O_2 consumption a R/ETS ratio of 12.8 mol O_2/mol INT-F was used (Martínez-García et al. 2009). This empirical relationship was stablished for nonaxenic cultures of *Isochrysis galbana* with different bacterial to algal biomass ratio using the same incubation time for measuring oxygen consumption and INT-F formation (Martínez-García et al. 2009). A large set ($n = 72$) of field experiments using a wide range of marine microbial planktonic communities (sampled in oligotrophic North Atlantic Subtropical Grye and in mesotrophic/eutrophic NW Iberian Peninsula) was also performed to evaluate if the oxygen consumption to INT reduction ratio (R/ETS) obtained with nonaxenic algal cultures in the lab would also hold for different natural samples. In these field experiments, the incubation time was not the same for measuring oxygen consumption and INT-F formation. The mean [$\pm$SE] R/ETS ratio obtained for natural oligotrophic waters was 12.5 [$\pm$2.0] ($p < 0.001$, $n = 45$) (Martínez-García et al. 2009). This R/ETS ratio for oligotrophic communities was not significantly different from that derived from algal cultures. Furthermore, a constant relationship between oxygen consumption and INT-F formation rates was found over a two orders of magnitude range of respiration rates (0.1–8.3 μM O_2 d^{-1}) (Martínez-García et al. 2009). Although the R/ETS ratio may vary between different microbial communities and this should be taken into account when predicting MCR from *in vivo* ETS activity data, the use of a mean ratio should not introduce large uncertainty (as mentioned above ca. 16% in the case of oligotrophic communities) in the estimation of oxygen consumption rates.

Ancillary data

As sampling for this investigation was embedded in the research programs of the long-term monitoring of Station ALOHA, HOT, and the C-MORE, additional core measurements were available (Table 1). The measurement protocols are available at: http://hahana.soest.hawaii.edu/hot/.

Statistical analysis

T-test analyses were performed to compare different samples and a significance level of $p < 0.05$ was used (Sokal and Rohlf 1995). The effect of sampling depth and sampling time on MCR was assessed by analysis of variance (ANOVA) and permutational multivariate analysis of variance (PERMANOVA) and a significance level of $p < 0.05$ was used (Anderson 2001).

Results

Study site

Mixed layer depths (MLD), calculated using the 0.125 kg m^{-3} potential density offset from the surface (Suga et al. 2004), ranged from 37 m to 118 m (Table 1). We defined periods of strong stratification of the water column (MLD $\geq$ 60 m; 60 m is the climatological mean MLD at Station ALOHA from 1989 to 2012 [http://hahana.soest.hawaii.edu/hot/hot-dogs/interface.html]): November 2011, March, May, August, and October 2012; vs. periods of weak stratification (MLD $>$ 60 m): December 2011, January, April, and June 2012 (Table 1).

Vertical distribution of microbial community respiration (MCR)

Nine vertical profiles from 0 m to 200 m of total MCR, respiration in the 0.2–0.8 μm size fraction (0.2–0.8 μm MCR hereafter), respiration in the size fraction >0.8 μm (>0.8 μm MCR hereafter) and the respiration in the size-fraction >0.8 μm relative to respiration in the 0.2–0.8 μm size-fraction (0.8/0.2 μm MCR hereafter) were measured at Station ALOHA on approximately monthly intervals (Fig. 1). The statistical analysis showed that sampling depth had a significant effect on total MCR, 0.2–0.8 μm MCR, >0.8 μm MCR and 0.8/0.2 μm MCR ($p < 0.05$, PERMANOVA). Volumetric (mmol O_2 m^{-3} d^{-1}) total MCR in the upper euphotic zone (0–100 m) (mean [$\pm$SE] value 0.89 [$\pm$0.05] mmol O_2 m^{-3} d^{-1}) was significantly higher ($p < 0.001$, $n = 36$) than in the lower euphotic zone (100–200 m) (0.52 [$\pm$0.05] mmol O_2 m^{-3} d^{-1}) (Table 2; Fig. 1). In the upper euphotic zone

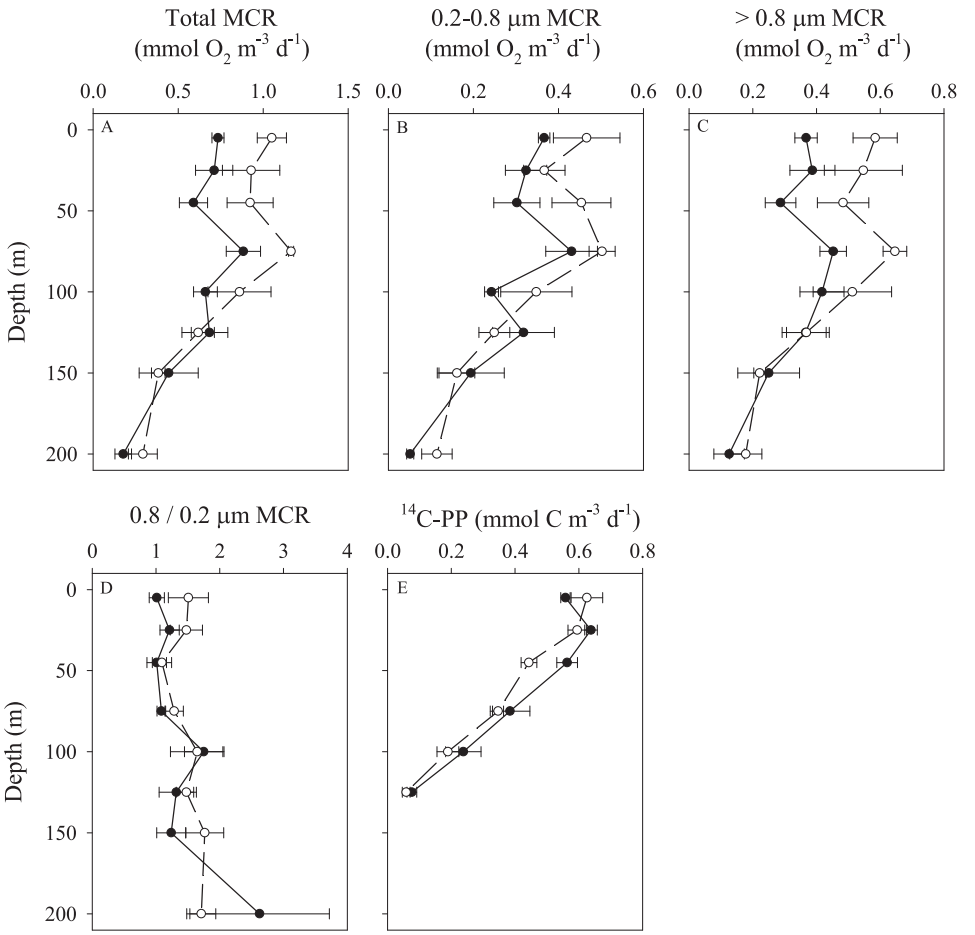


Fig. 1. Depth distribution of the means of (A) total microbial community respiration (Total MCR), (B) respiration in the 0.2–0.8 μm size-fraction (0.2–0.8 μm MCR), (C) respiration in the size-fraction $>$ 0.8 μm ($>$ 0.8 μm MCR), (D) respiration in the $>$ 0.8 μm size-fraction relative to respiration in the 0.2–0.8 μm size-fraction (0.8/0.2 μm MCR), and (E) ^{14}C-based primary production (^{14}C-PP) for the periods with MLD $>$ 60 m (black symbols) and $\leq$ 60 m (white symbols). Error bars represent the standard error; where error bars are not visible, they are smaller than the size of the symbol.

0.2–0.8 μm MCR (0.41 [$\pm$0.02] mmol O_2 m^{-3} d^{-1}) was significantly higher ($p < 0.001$, $n = 36$) than in the lower euphotic zone (0.21 [$\pm$0.05] mmol O_2 m^{-3} d^{-1}) (Table 2; Fig. 1B). The $>$0.8 μm MCR in the upper euphotic zone (0–100 m) (0.48 [$\pm$0.03] mmol O_2 m^{-3} d^{-1}) was also significantly higher ($p < 0.001$, $n = 36$) than the rates in the lower euphotic zone (0.31 [$\pm$0.03] mmol O_2 m^{-3} d^{-1}) (Table 2; Fig. 1C). The 0.8/0.2 μm MCR in the upper euphotic zone (1.22 [$\pm$0.07]) was significantly lower ($p < 0.05$, $n = 36$) than in the lower euphotic zone (1.68 [$\pm$0.17]) (Table 2; Fig. 1D).

Temporal variability of MCR in the euphotic zone at Station ALOHA was also investigated. The statistical analysis showed that the interaction between sampling time (i.e., month) and sampling depth had a significant effect on total MCR, 0.2–0.8 μm, $>$0.8 μm and 0.8/0.2 μm MCR ($p < 0.05$, PERMANOVA). However, sampling time had a significant effect only on 0.8/0.2 μm MCR ($p < 0.05$, PERMANOVA). We compared total and size-fractionated MCR during periods of strong stratification of the water column (MLD $\leq$ 60 m) to periods of weak stratification (MLD $>$ 60 m) (Table 1). During the more stratified periods, total MCR in the upper (0–100 m) euphotic zone (1.01 [$\pm$0.06] mmol O_2 m^{-3} d^{-1}) was significantly higher ($p < 0.01$) than the rates measured during periods of weak stratification (0.73 [$\pm$0.01] mmol O_2 m^{-3} d^{-1}; Table 2; Fig. 1A). Similarly, 0.2–0.8 μm MCR and $>$0.8 μm MCR in the upper (0–100 m) euphotic zone during the more stratified periods (0.45 [$\pm$0.03] and 0.56 [$\pm$0.04] mmol O_2 m^{-3} d^{-1}, respectively) were significantly higher ($p < 0.05$) than the rates measured during less stratified periods (0.36 [$\pm$0.03] and 0.37 [$\pm$0.03] mmol O_2 m^{-3} d^{-1}, respectively; Table 2; Fig. 1B,C). However, 0.8/0.2 μm MCR in the upper euphotic zone was not significantly different ($p > 0.05$) between the two opposing conditions of water column stratification (1.22 [$\pm$0.07]); Table 2; Fig. 1D). Total MCR, 0.2–0.8 μm and $>$0.8 μm MCR achieved maximum values at 75 m for both conditions of the water column stratification (Fig. 1A–C).

Table 2. Mean (± standard error) volumetric (mmol O_2 m^{-3} d^{-1}) values of: respiration in the 0.2–0.8 μm size-fraction (0.2–0.8 μm MCR), respiration in the > 0.8 μm size-fraction (> 0.8 μm MCR), total microbial community respiration (Total MCR = 0.2–0.8 μm MCR + > 0.8 μm MCR) and respiration in the > 0.8 μm size-fraction relative to respiration in the 0.2–0.8 μm size-fraction (0.8/0.2 μm MCR = > 0.8 μm MCR / 0.2–0.8 μm MCR) in the upper and lower euphotic zone for the periods with MLD > 60 m and ≤ 60 m. Monthly, daily, and hourly variabilities of volumetric rates at 25 m and monthly variability of depth-integrated (0–200 m) rates (mean and standard error) are also shown. Values in brackets are the corresponding range of values

	Variable			
	0.2–0.8 μm MCR (mmol O_2 $m^{-3}d^{-1}$)	**> 0.8 μm MCR (mmol O_2 $m^{-3}d^{-1}$)**	**Total MCR (mmol O_2 $m^{-1}d^{-1}$)**	**0.8 / 0.2 μm MCR**
Upper euphotic zone (0–100 m)				
All	0.41 ± 0.02 (0.16–0.72)	0.48 ± 0.03 (0.13–0.98)	0.89 ± 0.05 (0.31–1.54)	1.22 ± 0.07 (0.52–2.48)
MLD ≤ 60m	0.45 ± 0.03 (0.20–0.72)	0.56 ± 0.04 (0.13–0.98)	1.01 ± 0.06 (0.33–1.54)	1.34 ± 0.12 (0.52–2.48)
MLD > 60m	0.36 ± 0.03 (0.16–0.61)	0.37 ± 0.03 (0.15–0.61)	0.73 ± 0.01 (0.31–1.18)	1.08 ± 0.07 (0.54–1.56)
Lower euphotic zone (100–200 m)				
All	0.21 ± 0.05 (0.03–0.68)	0.31 ± 0.03 (0.03–0.87)	0.52 ± 0.05 (0.08–1.43)	1.68 ± 0.17 (0.51–6.41)
MLD ≤ 60m	0.22 ± 0.03 (0.05–0.68)	0.32 ± 0.05 (0.09–0.87)	0.54 ± 0.07 (0.15–1.43)	1.65 ± 0.15 (0.51–3.14)
MLD > 60m	0.20 ± 0.04 (0.03–0.55)	0.29 ± 0.05 (0.03–0.66)	0.49 ± 0.07 (0.08–0.99)	1.73 ± 0.33 (0.54–6.41)
Monthly variability at 25 m				
	28 ± 7% (1–67%)	40 ± 9% (3–97%)	32 ± 8% (2–85%)	30 ± 6% (6–74%)
Daily variability at 25 m				
	41 ± 6% (4–85%)	26 ± 5% (3–72%)	29 ± 4% (2–58%)	46 ± 10% (1–185%)
Hourly variability at 25 m				
	33 ± 7% (8–96%)	21 ± 3% (9–41%)	15 ± 4% (1–41%)	48 ± 9% (11–134%)
Monthly variability of depth–integrated (0–200 m) rates				
	14 ± 4% (1–42%)	20 ± 5% (1–51%)	15 ± 4% (1–36%)	30 ± 6% (6–74%)

MCR was not correlated with chlorophyll *a* (Chl *a*) concentration or ^{14}C-based primary productivity (^{14}C-PP) ($p > 0.05$, $n = 72$). ^{14}C-PP was relatively constant in the first 25 m and decreased rapidly at greater depths (Fig. 1E). Consequently, ^{14}C-PP rates were significantly higher ($p < 0.001$) in the upper (6.20 [± 0.29] mg C m^{-3} d^{-1}) than in the lower euphotic zone (1.65 [± 0.32] mg C m^{-3} d^{-1}). The decrease in ^{14}C-PP with depth within the euphotic zone was more pronounced than in MCR (Fig. 2). There was an approximately 30 m offset between the depths where cumulative ^{14}C-PP and cumulative MCR reached 50% of their respective 0–200 m integrated (mmol O_2 m^{-2} d^{-1}) totals (~ 45 m vs. ~ 75 m; Fig. 2).^{14}C-PP was similar during both conditions of stratification ($p > 0.05$) (Fig. 1E).

Monthly variability in MCR was calculated as the difference between the measured value at 25 m for a given cruise and the 25 m mean value from November 2011 to October 2012 (i.e., % variability = [(monthly value − mean) × 100/ mean]). This depth was chosen to be representative of euphotic zone respiration because a significant relationship (r^2 = 0.61, $p < 0.05$, $n = 9$) was found in the nine cruises between the volumetric (mmol O_2 m^{-3} d^{-1}) MCR at 25 m and depth-integrated (mmol O_2 m^{-2} d^{-1}) values in the euphotic zone (0–200 m). Mean monthly variabilities of total

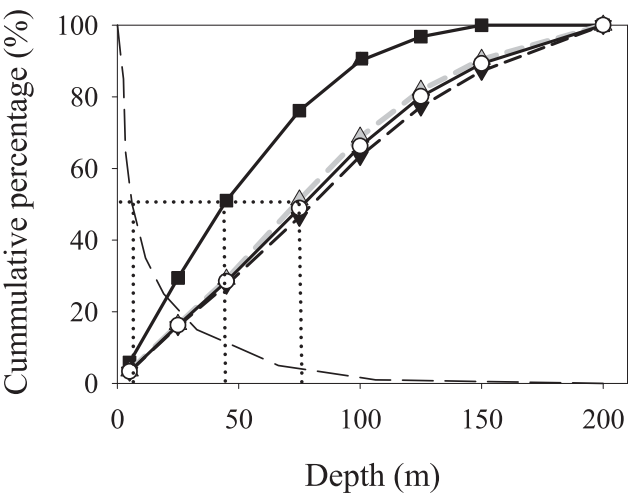


Fig. 2. Cumulative percentage of depth-integrated total MCR (white circles), respiration in the 0.2–0.8 μm size-fraction (0.2–0.8 μm MCR) (grey triangles), respiration in the size-fraction > 0.8 μm (> 0.8 μm MCR) (black triangles) and ^{14}C-based primary production (^{14}C-PP) (black squares) relative to the full euphotic zone (0–200 m) depth-integrated value. Dashed line represents the percentage of photosynthetic active radiation relative to the surface. Dotted lines mark the depths at which the percentage is 50% for each variable.

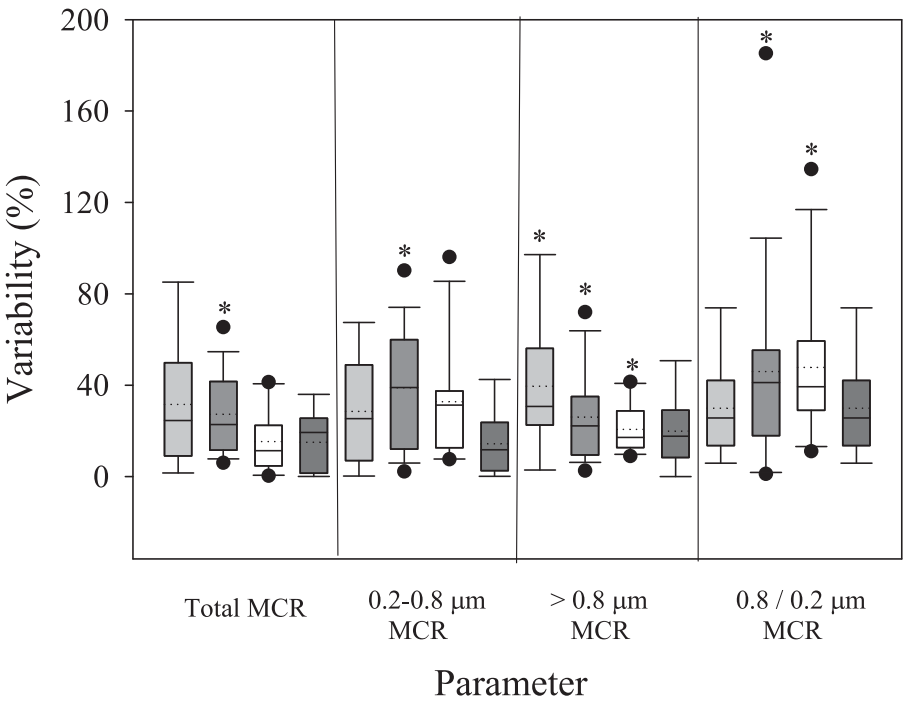


Fig. 3. Monthly (light grey box), daily (grey box), and hourly (white box) variability at 25 m and monthly variability of depth-integrated (0–200 m) MCR (dark grey box) of MCR, respiration in the 0.2–0.8 μm size-fraction (0.2–0.8 μm MCR), respiration in the >0.8 μm size-fraction (>0.8 μm MCR) and respiration in the size-fraction >0.8 μm relative to respiration in the 0.2–0.8 μm size-fraction (0.8/0.2 μm MCR) at Station ALOHA. Variability was calculated as the difference between the specific value and the mean value for the studied period relative to the average value (i.e., % Daily variability = [(Daily value − mean)*100/mean]). Box boundaries indicate the 25th and 75th percentiles. Error bars above and below the box indicate the 90th and 10th percentiles. Solid circles represent outliers. Dotted and solid line within the boxes represent the mean and the median, respectively. Asterisks represent variability significantly different ($p<0.05$) from methodological variability of the respiration method (i.e., variability between replicates).

MCR and 0.2–0.8 μm, >0.8 μm and 0.8/0.2 μm MCR at 25 m were 32%, 28%, 40%, and 30%, respectively (Table 2; Fig. 3).

Daily and hourly variability of MCR

During a 17-day period (23 August–08 September 2012), 25 m water samples were taken every 24 h to study day-to-day variability of MCR (Fig. 4). During this period the mean [$\pm$SE] total MCR, 0.2–0.8 μm MCR, and >0.8 μm MCR were 0.84 [$\pm$0.02], 0.36 [$\pm$0.04] and 0.48 [$\pm$0.04] mmol O_2 m^{-3} d^{-1}, respectively (Fig. 4). Mean [$\pm$SE] 0.8/0.2 μm MCR was 1.71 [$\pm$0.26]. Daily total and >0.8 μm MCR variabilities were 29% and 26%, respectively (Table 2; Fig. 3). However, 0.2–0.8 μm MCR was more variable on a daily basis and the percentage change relative to the mean for the entire period was on average 41% (Table 2; Fig. 3). Daily 0.8/0.2 μm MCR variability was 46% (Table 2; Fig. 3).

To study hour-to-hour variability of MCR at Station ALOHA 25 m samples were taken every four hours during a 44 h period between 31 August and 01 September 2012 (Fig. 5). During this period total, 0.2–08 μm and >0.8 μm MCR were 0.048 [$\pm$0.003], 0.024 [$\pm$0.002] and 0.024 [$\pm$0.002] mmol O_2 m^{-3} h^{-1}, respectively. Mean [$\pm$SE] 0.8/0.2 μm MCR was 1.26 [$\pm$0.21]. Total and >0.8 μm MCR hourly variabilities were 15% and 21% respectively over the observation period (Table 2; Fig. 3). However, 0.2–0.8 μm and 0.8/0.2 μm MCR hourly variabilities were 33% and 48% respectively. Changes in total MCR were coupled with changes in 0.2–0.8 μm MCR (Fig. 5). Statistical analysis showed that sampling time (i.e., local time) did not have a significant effect on total, 0.2–0.8 μm, >0.8 μm and 0.8/0.2 μm MCR ($p>0.05$, ANOVA). Mean values at night were not significantly different ($p>0.05$) from mean values during the day.

Depth integrated rates of MCR

Depth-integrated (0–200 m, mmol O_2 m^{-2} d^{-1}) total MCR rates ranged from 107.1 mmol O_2 m^{-2} d^{-1} to 183.4 mmol O_2 m^{-2} d^{-1} (mean value [$\pm$SE] was 134.8 [$\pm$11.8] mmol O_2 m^{-2} d^{-1}; Fig. 6). Depth-integrated (0–200 m) 0.2–0.8 μm and >0.8 μm MCR ranged from 52.1 mmol O_2 m^{-2} d^{-1} to 84.2 mmol O_2 m^{-2} d^{-1} (59.1 [$\pm$3.9] mmol O_2 m^{-2} d^{-1}) and from 50.8 mmol O_2 m^{-2} d^{-1} to 114.2 mmol O_2 m^{-2} d^{-1} (75.7 [$\pm$6.2] mmol O_2 m^{-2} d^{-1}), respectively (Fig. 6). Depth-integrated (0–200 m) total MCR, 0.2–0.8 μm MCR and >0.8 μm MCR were not significantly different ($p>0.05$) during periods of stronger (147.6 [$\pm$2.7], 63.2 [$\pm$5.6] and 84.3 [$\pm$9.3] mmol O_2 m^{-2} d^{-1}, respectively) and weaker stratification (118.9 [$\pm$5.5], 53.9 [$\pm$3.7] and 65.0 [$\pm$3.0] mmol O_2

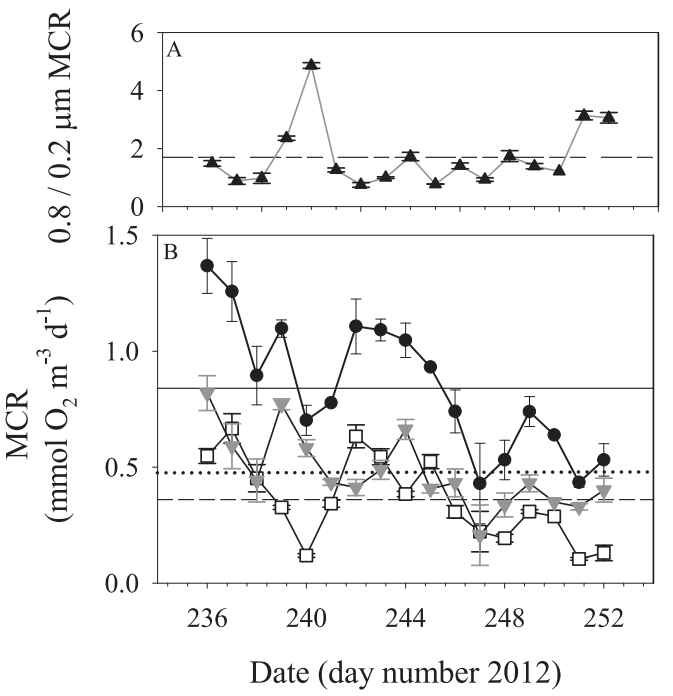


Fig. 4. Daily variability at 25 m at Station ALOHA of (A) respiration in the > 0.8 μm size-fraction relative to respiration in the 0.2–0.8 μm size-fraction (0.8/0.2 μm MCR) (black up-triangles) and (B) total MCR (black circles), respiration in the 0.2–0.8 μm size-fraction (0.2–0.8 μm MCR) (white squares), and respiration in the size-fraction > 0.8 μm (> 0.8 μm MCR) (grey down-triangles) measured at 25 m at Station ALOHA from 23 August 2012 to 08 September 2012. Error bars represent the standard error; where error bars are not visible, they are smaller than the symbol size. Dashed line in (A) represents the mean 0.8/0.2 μm MCR. Solid, dashed and dotted lines in (B) represent the mean MCR, 0.2–0.8 μm MCR and > 0.8 μm MCR, respectively for the studied period.

m^{-2} d^{-1}, respectively). Depth-averaged (0–200 m) 0.8/0.2 μm MCR ranged from 0.9 to 2.2 (mean value [± SE] was 1.4 [± 0.1]) and was similar during periods of opposing conditions of water column stratification (Fig. 6). Depth-integrated (0–200 m) MCR was not correlated with integrated Chl *a* concentration or with ^{14}C-PP ($p > 0.05$, $n = 72$). Depth-integrated (0–200 m) MCR monthly variabilities of total, 0.2–0.8 μm, > 0.8 μm and 0.8/0.2 μm MCR, were 15%, 14%, 20%, and 30% respectively (Table 2; Fig. 3).

Discussion

Highest respiration rates were found in the upper euphotic zone (0–100 m) at Station ALOHA consistent with higher solar energy flux, microbial biomass, ^{14}C-PP, nutrient uptake, and particle concentrations. The local peak in MCR at 75 m corresponds to biomass increases of several populations (e.g., heterotrophic bacteria, *Prochlorococcus*, *Synechococcus* and picoeukaryotes) reported by the HOT program (http://hahana.soest.hawaii.edu/hot/hot-dogs/), but the reasons for this feature are presently unknown. Also, dissolved organic phosphorus hydrolysis rates were reported to be highest at this depth at Station ALOHA (Duhamel et al. 2011).

Our MCR depth profiles did not match the observations of Williams et al. (2004). They reported a maximum at 25 m depth, but also a minimum in net oxygen production at 75 m which is consistent with the local MCR maximum reported herein. MCR depth profiles varied independently of ^{14}C-PP; ^{14}C-PP was highest in the first 25 m and sharply decreased at greater depths (Letelier et al. 1996) while total, 0.2–0.8 μm and > 0.8 μm MCR all exhibited less pronounced decreases with depth (Fig. 2). The uncoupling between ^{14}C-PP and MCR in the water column has been previously reported in oligotrophic systems (Arístegui and Harrison 2002; Morán et al. 2007), but the cause is unknown. MCR includes the respiratory activities of photolithoautotrophs, so we had anticipated that ^{14}C-PP and MCR might be correlated in space and time, which was not the case. Furthermore, the production of labile dissolved organic matter to support the respiratory activities of chemoorganoheterotrophs should be coupled to ^{14}C-PP. The decoupling of MCR and ^{14}C-PP remains enigmatic.

In the present study, higher MCR in the upper euphotic zone was found during periods with more stratified water column. However, ^{14}C-PP was not significantly different between stratified and mixed conditions. This result is another indication of a decoupling between these major metabolic processes. Although higher temperature during stratified periods could differentially affect MCR and ^{14}C-PP, the difference in surface temperatures between the two periods (i.e., ca. 1°C) is insufficient to explain the difference in respiration rates considering a Q10 value of 2 for microbial respiration (Robinson and Williams 1993).

Our results suggest that at Station ALOHA both the > 0.8 μm and the 0.2–0.8 μm size fractions play important roles in the consumption of oxygen. The average relationship between the > 0.8 μm and the 0.2–0.8 μm size fractions (i.e., 0.8/0.2 μm MCR) was 1.4 ± 0.4. Higher 0.8/0.2 μm MCR were found in the upper than lower euphotic zone, particularly during periods of weaker stratification, this probably related to aggregation in the form of sinking particles in deeper layers. These size fractions are somewhat arbitrary for environments like Station ALOHA because size alone cannot be used to distinguish specific taxonomic or trophic groups. Also, most of the "photolithoautotrophs" at Station ALOHA are bacteria, and have a size distribution that overlaps with the chemoorganoheterotrophic bacteria. We estimated picophytoplankton respiration using measured ^{14}C-PP and the assumption of Marra and Barber (2004) that respiration by photolithoautotrophs equals twice the overnight loss of organic carbon (assuming dark respiratory losses amount to 20% of ^{14}C-PP, Geider 1992). Applying this assumption to historical primary production data at Station ALOHA (http://hahana.soest.hawaii.edu/hot/hot-dogs/), the depth integrated

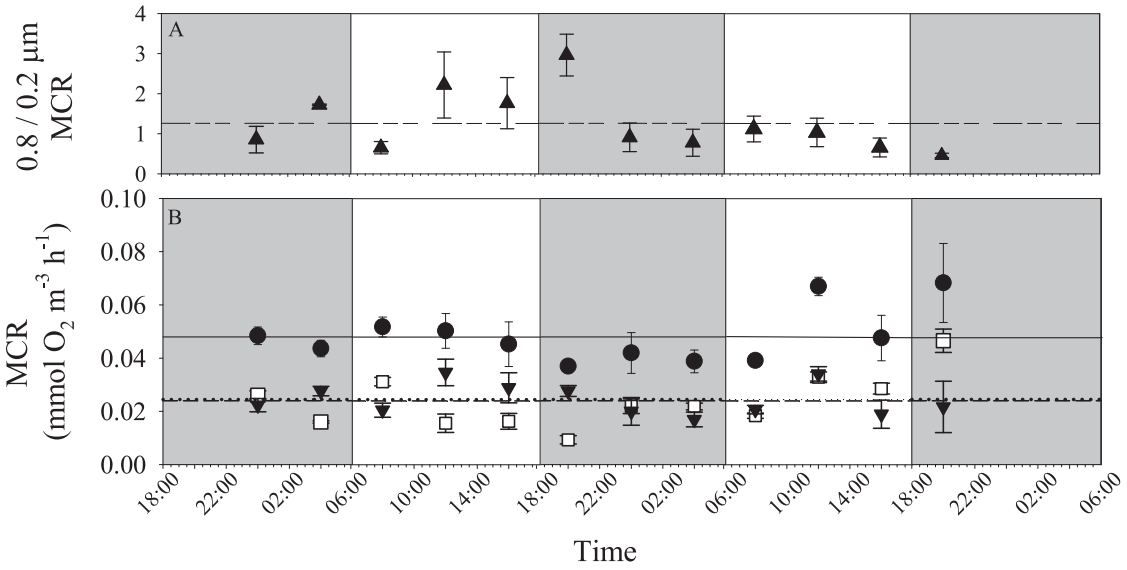


Fig. 5. Hourly variability at 25 m at Station ALOHA of (A) respiration in the size-fraction > 0.8 μm relative to respiration in the 0.2–0.8 μm size-fraction (0.8/0.2 μm MCR) (black up-triangles) and (B) Total MCR (black circles), respiration in the 0.2–0.8 μm size-fraction (0.2–0.8 μm MCR) (white squares), and respiration in the size-fraction > 0.8 μm (> 0.8 μm MCR) (black down-triangles) measured at 25 m at Station ALOHA from 00:00 on 31 August to 20:00 on 01 September 2012. Error bars represent the standard error; where error bars are not visible, they are smaller than the symbol size. Dashed line in (A) represents the mean 0.8/0.2 μm MCR. Solid, dashed and dotted lines in (B) represent the mean MCR, 0.2–0.8 μm MCR and > 0.8 μm MCR, respectively for the studied period.

(0–200 m) picophytoplankton respiration would be 21.5 ± 1.0 mmol O_2 m^{-2} d^{-1}. During the present study, estimated picophytoplankton respiration (following Marra and Barber 2004) was 25.5 ± 0.9 mmol O_2 m^{-2} d^{-1} (approximately $20.2 \pm 1.1\%$ of total MCR). Note that December (HOT 238) and March (HOT 240) cruises have been excluded from these calculations because no ^{14}C-PP data are available. Bacterivore respiration was calculated as a function of bacterial production (BP) (Straile 1997; Nagata 2000; Zubkov et al. 2000; Robinson et al. 2002). Assuming a mean value of BP for the euphotic zone at Station ALOHA of 0.45 mg C m^{-3} d^{-1}(Church et al. 2004), the contribution of bacterivores to MCR would be $4.0 \pm 0.2\%$ of total MCR, or 5.1 ± 0.1 mmol O_2 m^{-2} d^{-1}. The remainder of the MCR, approximately $75.7 \pm 1.2\%$ (or 98.4 ± 6.5 mmol O_2 m^{-2} d^{-1}) is assumed to be due to chemo-organoheterotrophic bacteria, both free-living and those attached to suspended particles.

We found that variability of MCR at 25 m was different for the different size-fractions studied. While total MCR and > 0.8 μm MCR were more variable over longer time scales (i.e., monthly variability), 0.2–0.8 μm and 0.8/0.2 μm MCR were more variable in shorter time scales [i.e., daily and hourly variability (Fig. 3)]. This finding suggests that volumetric (mmol O_2 m^{-3} d^{-1}) total MCR at Station ALOHA is temporally variable, although changes are not expected over very short time scales (i.e., hours). Conversely, the size-distribution of MCR at Station ALOHA varies on the short-term (hours to days), and less on the annual time scales. On the short-term, 0.2–0.8 μm MCR was more variable than total MCR, and changes in total MCR were associated with changes in the size spectrum of MCR. This indicates that microorganisms in the 0.2–0.8 μm size fraction at Station ALOHA are more dynamic than cells > 0.8 μm on both daily and hourly time scales (Fig. 3). These results indicate that biomass and/or activity of large cells (i.e., larger bacteria, phytoplankton, and protozoa) and particle-attached microbes are less variable on a daily basis that those of smaller cells at Station ALOHA. This is consistent with the paradigm of free-living heterotrophic bacteria as rapid responders to both bottom-up and top-down controls in the open ocean that may operate over hourly and daily time scales (Azam et al. 1983; Weisse and Scheffel-Möser 1991; Cotner and Biddanda 2002; Sharma et al. 2013). In the present study, no significant differences were found between mean daytime and mean nighttime MCR, either for the total microbial community or the different size fractions (Fig. 6). It is also important to note here that respiration rates were always measured in dark conditions, and that this experimental design may have altered the potential difference between daytime and nighttime MCR. Recently, Ottesen et al. (2014) found daytime peaks in messenger RNA transcripts associated with respiration genes in the most abundant bacterioplankton groups at Station ALOHA (*Prochlorococcus*, SAR11, SAR 116 and *Roseobacter*).

Depth-integrated MCR showed low variability in both size-fractions and over the temporal scale studied (Fig. 3) suggesting a relative constancy of depth-integrated MCR throughout the year in the euphotic zone at Station ALOHA.

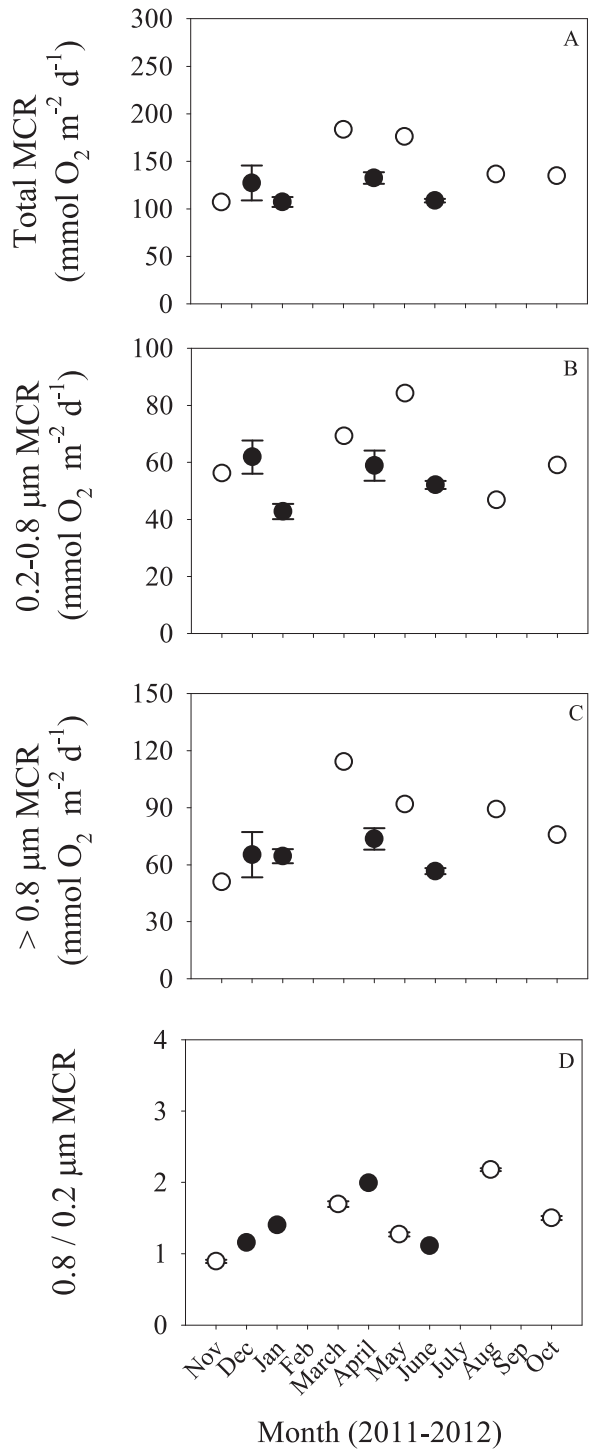


Fig. 6. Euphotic zone depth-integrated (0–200 m) rates of (A) total MCR, (B) respiration in the 0.2–0.8 μm size-fraction (0.2–0.8 μm MCR), (C) respiration in the size-fraction > 0.8 μm (> 0.8 μm MCR), and (D) respiration in the size-fraction > 0.8 μm relative to respiration in the 0.2–0.8 μm size-fraction (0.8/0.2 μm MCR) for the periods with MLD > 60 m (black symbols) and ≤ 60 m (white symbols). Average values and standard errors are presented, where error bars are not visible, they are smaller than the symbol size. Note that different scales are used.

Our dataset indicates that higher depth-integrated (0–200 m) MCR is associated with periods with a more stratified water column (i.e., MLD ≤ 60 m) at Station ALOHA and this is mostly due to increased respiration in the upper (0–100 m) euphotic zone. This may be a result of an increase in light available for photosynthesis or due to an increase in nutrients or organic matter derived from the atmosphere. However, ^{14}C-PP production rates were not elevated in the upper 0–100 m during the more stratified periods. Alternatively, MCR may be fuelled by in situ dissolved organic matter production during photosynthesis that would not have been detected in our study. No clear seasonal changes in depth-integrated (0–200 m, mmol O_2 m^{-2} d^{-1}) MCR were found (Table 1). This result conflicts with Williams et al. (2004), who reported a higher integrated production at Station ALOHA during the summer period. In the present study, as well as in Williams et al. (2004), no relationship was found between integrated production measured by the ^{14}C-PP and MCR from November 2011 to October 2012, possibly for reasons presented earlier (i.e., differences in the DOC produced by autotrophs that is not accounted for with ^{14}C-PP, the availability of essential growth factors and an increase in temperature during stratification periods may fuel MCR).

The lack of relationship found between integrated ^{14}C-PP and MCR is in accordance with previous studies reporting an uncoupling between primary production and respiration processes in the oligotrophic ocean (Arístegui and Harrison 2002; Robinson et al. 2002; Morán et al. 2007). Karl et al. (2003) suggested a decoupling between time-varying PP and a more constant MCR at Station ALOHA. Only a few studies (e.g., Williams and Purdie 1991; Williams et al. 2004) have investigated MCR with a time and depth coverage sufficient to constrain net community production (NCP; where NCP = GPP − MCR). Based on 24-h in situ bottle incubation measurements of O_2, Williams et al. (2004) found negative NCP (i.e., MCR > GPP) throughout the year at Station ALOHA (i.e., NCP = -9 ± 1.7 mol O_2 m^{-2} yr^{-1}). However, Williams et al. (2004) favored previous geochemical findings indicating that the euphotic zone at Station ALOHA is net autotrophic (Emerson et al. 1997) rather than their own in vitro O_2 flux measurements. They argued that this discrepancy is due to the difficulty of reproducing the light field, and the intermittency of production compared to respiration, both leading to underestimation of GPP. It is interesting to mention here that average 0–150 m depth-integrated respiration rates found in the present study (117 ± 8 mmol O_2 m^{-2} d^{-1}) are higher than those reported by Williams et al. (2004) (85 ± 5 mmol O_2 m^{-2} d^{-1}) while similar annual average^{14}C-PP were measured for both time periods. In the present study, integrated MCR from 0 m to 200 m depth always exceeded the production of O_2 extrapolated from ^{14}C-PP and assuming a photosynthetic quotient of 1.13 ($\Delta O_2/\Delta CO_2$, Laws 1991). This comparison yields an O_2 deficit

throughout the year at Station ALOHA (NCP = -71.3 ± 8.6 mmol O_2 m^{-2} d^{-1}), with lower NCP values during more stratified periods (-84.1 ± 9.7 mmol O_2 m^{-2} d^{-1}) than during periods with a less stratified water column (-54.2 ± 3.4 mmol O_2 m^{-2} d^{-1}). Our data suggest that GPP may be significantly underestimated by the ^{14}C technique. It can also be argued that MCR presented here has been overestimated (*see* discussion on conversion factor in Methods section). Still, our MCR are in the range of those found by Williams et al. (2004) using bottle incubation measurements of O_2 and the oxygen consumption budgets presented here agree with previous estimates of GPP at Station ALOHA (Quay et al. 2010).

We have presented a unique dataset on temporal and spatial variability of MCR at Station ALOHA. Both size fractions of microorganisms (> 0.8 μm and 0.2–0.8 μm) play an important role in O_2 consumption at Station ALOHA. Short-term variability in MCR is evident, particularly in the 0.2–0.8 μm size-fraction, but a relative constancy of depth-integrated MCR throughout the year in the euphotic zone at Station ALOHA suggests the importance of scale in reaching any conclusions on the metabolic balance of the ecosystem. Periods of stratified water column conditions at Station ALOHA led to higher MCR but lower NCP based on a comparison of ^{14}C-PP and MCR. These observations allow us to calculate a lower limit of GPP during the 1-year observation period (November 2011–October 2012). By converting our MCR from O_2 to carbon-based currency using a respiratory quotient of 0.89 (ΔCO_2 : ΔO_2, Williams and del Giorgio 2005) and assuming steady-state on an annual time scale, the sum of measured MCR in the euphotic zone plus particulate carbon export from the euphotic zone sets a lower limit of 45 mol C m^{-2} yr^{-1} for GPP at this oligotrophic site, a value that exceeds the reported ^{14}C-based estimate (Karl et al. 2002) by at least a factor of 2.5. Carbon assimilated into higher trophic levels and exported by means other than passively sinking particulate matter (e.g., actively migrating zooplankton or buoyant particles) and dissolved organic matter diffusion would increase this estimate. Photosynthetic production of organic carbon is generally assumed to be the primary process of solar energy capture in marine planktonic systems and is the basis of the ocean's food web and the biological carbon pump, influencing eventually fish and deep-sea benthic community production, carbon sequestration and CO_2 and O_2 exchanges with the atmosphere. Therefore, its assessment is crucial if we aim at understanding oxygen and carbon cycles in the ocean and the atmosphere. Oligotrophic regions like Station ALOHA represent some of Earth's largest biomes and are sometimes considered to be the oceanic analogues of deserts where organic matter production is extremely low (Longhurst 2006). However, the data presented here indicate that autotrophic production may have been significantly underestimated and confirm that Station ALOHA is a remineralization-intensive ecosystem. The continued efforts of time series programs and the use of new methodologies may help constrain carbon, oxygen and nutrient budgets at these oligotrophic environments in the future.

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