Balancing organic carbon supply and consumption in the ocean’s interior: Evidence from repeated biogeochemical observations conducted in the subarctic and subtropical western North Pacific

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Abstract

The heterotrophic prokaryotic carbon demand (PCD) in the ocean’s interior often substantially exceeds (by up to two orders of magnitude) the amount of organic carbon supplied by sinking particulate organic carbon (POC). A hypothesis to explain this carbon imbalance proposes that some non-steady-state processes have not been considered in previous studies based on snapshot data. To test this hypothesis, we collected time-series (2.5 yr) data on sinking POC fluxes using moored sediment traps (trap deployment depths: 200 m, 500 m, and 4810 m) and compared them with the PCD data collected seasonally at two stations in the subarctic and subtropical western North Pacific. The POC supplies ($D_{POC}$) in the 200–500 m and 500–4810 m layers were estimated with a correction, when appropriate, for the non-steady-state effect arising from the change in the POC flux during the transit of POC between the upper and deeper traps. In the 200–500 m layer, $D_{POC}$ generally exceeded or equaled PCD. In contrast, in the 500–4810 m layer, PCD generally exceeded $D_{POC}$ by up to sevenfold. However, on a yearly basis, this carbon imbalance in the deeper layer decreased, with PCD balancing $D_{POC}$ within a factor of 2. Therefore, the enigma of the high PCD relative to the POC flux in deep water is partially resolved by assuming a temporal uncoupling between supply and consumption, which partly equilibrates the carbon budget over a longer (yearly) time scale.

Heterotrophic prokaryotes in the meso- and bathypelagic water masses of the ocean play a major role in regulating oceanic biogeochemical cycles (Nagata et al. 2010; Robinson et al. 2010; Herndl and Reintlherer 2013). They consume a substantial, albeit variable (50% to >100%), fraction of the organic carbon delivered from the sunlit layer to greater depths (i.e., export production, estimated to be in the order of 10 Pg C per annum globally; Dunne et al. 2007) and act as a major agent in the transformation and mineralization of organic matter in the ocean’s interior. These processes may affect the depth at which organic carbon is mineralized in the ocean (Buesseler and Boyd 2009), which may in turn affect the air–sea carbon balance and Earth’s climate (Kwon et al. 2009). Therefore, clarification of the mechanisms controlling the prokaryote-mediated carbon cycles in the deep oceanic water column is essential.

Existing data on the balance between organic carbon delivery and the heterotrophic prokaryotic carbon demand (PCD) in the meso- and bathypelagic layers are conflicting. Early studies conducted in the North Pacific generally found that the sinking particulate organic carbon (POC) fluxes were sufficient to meet PCD at depth (Cho and Azam 1988; Nagata et al. 2000). In contrast, Steinberg et al. (2008) found that PCD far exceeded (by up to 10-fold) the attenuation of sinking POC fluxes in the mesopelagic layer of the North Pacific. Similarly, PCD was much higher (up to 100-fold) than the sinking POC flux in the meso- and bathypelagic layers of the North Atlantic (Reintlherer et al. 2006) and western Arctic Ocean (Uchimiya et al. 2013). Yokokawa et al. (2013) collected extensive data on the full-depth distributions of
prokaryotic production along a latitudinal transect from the Southern Ocean to the subarctic Pacific and found that the sinking POC flux was generally sufficient to support the PCD in the mesopelagic layer, but too low to support the PCD in the bathypelagic layer. In the North Atlantic, Giering et al. (2014) found an excess supply in the upper mesopelagic layer (50–150 m) and a deficit in the lower mesopelagic layer (150–1000 m), although for the whole mesopelagic layer, prokaryotic carbon mineralization was equivalent to the sum of the organic carbon delivered by sinking POC and dissolved organic carbon (DOC) transport. These conflicting results have stimulated discussions about the errors and uncertainties inherent in the determination of organic carbon fluxes and PCD (Burd et al. 2010; Nagata et al. 2010), and spurred studies into the missing sources of organic carbon in the deep ocean (Reinthaler et al. 2010; Herndl and Reinthaler 2013) and the roles of mesozooplankton and other heterotrophs in organic carbon mineralization at depth (Steinberg et al. 2008; Robinson et al. 2010; Giering et al. 2014).

Investigators have also suggested that a temporal offset between organic carbon delivery and prokaryotic carbon consumption could be a source of error in the assessment of the deep-sea carbon budget (Hansell and Dukelow 2003; Nagata et al. 2010; Yokokawa et al. 2013). However, previous observations that PCD is high relative to the sinking POC supply are based on “snapshot” data collected on only one occasion or during a limited period (< 1 month) of study (Reinthaler et al. 2006; Steinberg et al. 2008; Yokokawa et al. 2013). If, for example, the PCD determined at a given time is supplemented by the organic carbon supplied in the past, presumably via the accumulation of semilabile DOC (Follett et al. 2014) and neutrally buoyant aggregates (Bochdansky et al. 2010) released from sinking POC and their subsequent consumption by prokaryotes, this could explain why PCD greatly exceeds the concurrent supply of sinking POC. In such a case, the PCD and POC flux would only be balanced over a longer (e.g., yearly) period, and not necessarily on a snapshot basis. Giering et al. (2017) have also recently suggested that the POC supply at depth estimated from the depth-dependent attenuation of the POC flux could have inherent errors associated with the assumption of a steady state. They suggested that the estimated POC supply is too low after a bloom (a period of declining POC flux) because the remnant POC from the bloom could diminish the attenuation of the flux, depending on the sinking speed and the mineralization rate of POC. Therefore, it is possible that the enigmatic high PCD relative to the sinking POC supply is a consequence of some non-steady-state processes involved in carbon supply and consumption in the deep ocean. However, testing this hypothesis has been hampered by the paucity of time-series dataset on the seasonal variability in both carbon supply and PCD at depth.

Here, we present data collected during the “K2S1 project,” which was conducted at two stations in the subarctic and subtropical western North Pacific. This project involved extensive time-series observations of the physical and biogeochemical properties of two disparate regions, in order to extend our understanding of the mechanisms controlling the carbon cycle in the western North Pacific (Honda et al. 2017). During the project, we examined the full-depth distributions of prokaryotic production and abundance on five occasions, covering a full seasonal cycle. These data were compared with time-series data on the sinking POC flux to investigate the balance in the organic carbon supply and PCD at depth. In this way, we examined the balance between sinking POC flux and microbial demand, with consideration of non-steady-state processes of the carbon cycle in the deep sea.

Materials and methods

Prokaryotic parameters

Samples were collected at two stations in the subarctic (47°N, 160°E; Sta. K2) and subtropical (30°N, 145°E; Sta. S1) regions of the western North Pacific (Fig. 1) during five cruises aboard the R/V Mirai undertaken between 2010 and 2012 (Table 1). Acid-washed 12 L Niskin bottles were used to collect seawater samples from 31 depths, ranging from 100 m below the surface to 10 m above the seafloor. The seawater samples used to determine prokaryotic production and abundance were transferred to acid-washed 1 L polycarbonate bottles.
(Nalgene, Thermo Fisher Scientific) and processed in a shipboard laboratory. Care was taken and gloves were worn throughout all sampling and processing procedures to minimize contamination.

Heterotrophic prokaryotic production was estimated using the incorporation rate of \(^{3}\)H-leucine (Kirchman 2001). Briefly, a 1.5 mL sample of seawater was transferred to a sterile tube and treated with 10 \(\mu\)L of \(^{3}\)H-leucine (NET460 or NET1166 [PerkinElmer]; specific activity, 4.0–6.1 TBq mmol\(^{-1}\); final concentration, 10 nmol L\(^{-1}\)). The samples were incubated in the dark at ambient temperature (± 2°C) for 2 h for samples from shallow waters (depth < 200 m) and for 24 h for samples from deep waters (> 200 m). After incubation, each sample was fixed by the addition of 80 \(\mu\)L of trichloroacetic acid (Wako) and then centrifuged at 16,000 \(\times\) g for 10 min (Kubota 1130 centrifuge, Kubota). The pellet was washed first with 1 mL of 5% trichloroacetic acid and then with 1 mL of 80% ethanol (Wako) and dried completely. In an onshore laboratory, 1 mL of scintillation cocktail (Ultima Gold, PerkinElmer) was added to the sample, and its radioactivity was determined with a liquid scintillation counter (either Tri-Carb 1500 [Packard], LS-6500SC [Beckman], or Tri-Carb 3110 TR [PerkinElmer], depending on the cruise). For each sample, three replicates and one killed control were prepared. The coefficient of variation of the disintegrations per minute (dpm) of the replicate samples averaged 13% ± 13% (n = 346). The data for six samples collected in the bathypelagic layer were not used in the subsequent analysis because the dpm of the killed control exceeded those of the replicate samples. The leucine incorporation rate was converted to prokaryotic production with a leucine-to-carbon conversion factor of 1.55 kg C mol\(^{-1}\) leucine (Simon and Azam 1989). To estimate PCD, we used a range of conversion factors reported in the literature (see “Estimation of PCD” section).

Prokaryotic abundance was determined with flow cytometry, according to the method of Yang et al. (2010). Briefly, a 2 mL seawater sample was placed in a sterile tube (Nalgene) and fixed by the addition of 100 \(\mu\)L of 0.02-\(\mu\)m-filtered 20% glutaraldehyde (final concentration, 1%; Wako). The fixed sample was refrigerated for 15 min, frozen in liquid nitrogen, and then stored in a deep freezer (−80°C) until analysis. The prokaryotic cells were counted in an onshore laboratory with a flow cytometer (FACSCalibur, BD) equipped with a 15 mW 488 nm laser and a standard filter set. The sample was stained with 10 \(\mu\)L of SYBR Green I (final concentration, 0.01% of the commercial stock; Invitrogen) and injected into the flow cytometer with reference beads (diameter 1 \(\mu\)m; Molecular Probes). Samples from shallower waters (depth < 200 m) were diluted 10-fold with Tris-EDTA (TE) buffer (10 mmol L\(^{-1}\) Tris-HCl, 1 mmol L\(^{-1}\) EDTA, pH 8.0; Nippon Gene) before staining. The background counts derived from the reference beads and TE buffer were < 0.1% of the total counts for the samples. The data were analyzed with the CellQuest (BD) software. The prokaryotic biomass was estimated by multiplying the prokaryotic abundance by a cell to carbon conversion factor of 12 fg C cell\(^{-1}\) (Fukuda et al. 1998).

### Hydrographic and other biogeochemical parameters

Data on the hydrographic and other biogeochemical parameters were obtained from the “K2S1 database” (https://ebcrpa.jamstec.go.jp/k2s1/en/index.html; last accessed 12 December 2017), managed by the Japan Agency for Marine-Earth Science and Technology (JAMSTEC). The seasonal variabilities in the surface-water temperature and the chlorophyll \(a\) (Chl \(a\)) concentration were analyzed based on the satellite data of Optimum Interpolation Sea Surface Temperature (OISST) and the Moderate Resolution Imaging Spectroradiometer (MODIS), respectively. Of the onboard observations, water temperature and salinity were determined with a conductivity-temperature-depth sensor (SBE 9plus, Sea-Bird Electronics), and photosynthetically active radiation (PAR) was determined with a PAR sensor (Satlantic). The base of the euphotic layer was defined as the depth at which PAR was equivalent to 0.5% of that at the sea surface. The mixed-layer depth was defined as the depth at which the water temperature deviated by > 0.2°C from the temperature at a depth of 10 m (Montégut et al. 2004). The DOC concentration was determined with the high-temperature-catalyzed oxidation method, using a total organic carbon analyzer (TOC-V, Shimadzu) (Wakita et al. 2016). The deviation of the values between duplicate samples was ± 1.0 \(\mu\)mol L\(^{-1}\), on average. The sinking POC flux was determined with moored time-series sediment traps (SMD26S-26, Nichiyu Giken Kogyo; Mark 7G-

### Table 1. Summary of the five cruises conducted at two observation stations.

| Cruise | Season  | Sampling date (mm/dd/yyyy) | Depth of the euphotic zone (m) | Mixed-layer depth (m) |
|--------|---------|---------------------------|--------------------------------|-----------------------|
|        |         | Sta. K2 | Sta. S1 | Sta. K2 | Sta. S1 | Sta. K2 | Sta. S1 |
| MR10-01| Winter  | 01/25/2010 | 01/31/2010 | 95 | 112 | 128 | 64 |
| MR10-06| Fall    | 10/24/2010 | 11/06/2010 | 62 | 94 | 46 | 41 |
| MR11-03| Spring  | 04/19/2011 | 04/29/2011 | 86 | 97 | 85 | 42 |
| MR11-05| Summer  | 06/30/2011 | 07/24/2011 | 49 | 93 | 25 | 19 |
| MR12-02| Summer  | 06/10/2012 | 06/26/2012 | 56 | 88 | 56 | 15 |
21, McLane), which were deployed at depths of 200 m, 500 m, and 4810 m between February 2010 and June 2012. The carbon contents of these samples were determined with an elemental analyzer (NC2500 [CE Instruments] or vario EL III [Elementar Analyseinstrumente GmbH]). Additional information regarding the sample collection, measurements, and data analysis is available from the K2S1 database.

Estimation of PCD

The PCD was calculated from the prokaryotic production based on the 3H-leucine incorporation rate. Because of the uncertainty in both the leucine-to-carbon conversion factor and the assumptions required for the calculation of PCD (Burd et al. 2010), we used two methods to determine PCD: with fixed conversion factors (Method 1), the conventional leucine-to-carbon conversion factor of 1.55 kg C mol⁻¹ leucine (Simon and Azam 1989) and a prokaryotic growth efficiency of 0.2 (del Giorgio and Cole 2000); and a Monte Carlo analysis (Method 2), in which the leucine-to-carbon conversion factors were previously reported empirical values of 0.13–0.85 kg C mol⁻¹ leucine (summarized by Baltar et al. 2010) and a growth efficiency of 0.01–0.24 (compiled by Mazuecos et al. 2015; see also Uchimiya et al. 2016). The leucine-to-carbon conversion factor and growth efficiency were randomly sampled from uniform distributions, and then PCD was calculated from the depth-integrated leucine incorporation rate and randomly sampled conversion factors. The PCD values estimated from 100,000 separate runs were used to calculate the average PCD.

Depth-dependent attenuation of sinking POC flux

The POC supply in the layer between 200 m and 500 m (APOC200–500) and that between 500 m and 4810 m (APOC500–4810) were estimated using the sinking POC fluxes (mg C m⁻² d⁻¹) at depths of 200 m (F200), 500 m (F500), and 4810 m (F4810) with a correction, when appropriate, for the error associated with changes in the sinking flux during the time required for the POC to transit through the water column from the upper to deeper traps (Giering et al. 2017). When F200 and F500 were in either the declining or increasing phase of their seasonal cycles at the time of sampling (see “Results” section), the quantity by which the flux attenuation in each depth layer is underestimated or overestimated (S, mg C m⁻² d⁻¹) was calculated as follows (Giering et al. 2017):

\[
S = z \left[ e^{-r(t)} - 1 \right] + \left[ 1 - e^{-r(t)} \right]
\]

where \(z\) is the slope of the linear increase or decrease in the sinking POC flux over time (range: 14–29 weeks), \(r\) is the mineralization rate (d⁻¹), and \(t\) (d) is the transit time of POC through the depth layers of interest, which is derived by dividing the thickness of the layer (z: 300 m [= 500–200] or 4310 m [= 4810–500]) by the sinking speed (v, m d⁻¹) of POC (i.e., \(t = z/v\)). The POC supply after correction for the non-steady-state process was calculated as follows: \(\Delta \text{POC}_{200} = F_{200} - S_{200-500}\) and \(\Delta \text{POC}_{500-4810} = F_{500} - S_{500-4810}\), where \(S_{200-500}\) and \(S_{500-4810}\) are the S values for the 200–500 m and 500–4810 m layers, respectively.

The non-steady-state model of Giering et al. (2017) requires assumptions for \(r\) and \(v\). We used \(r = 0.1\) and \(v = 50\) as the default parameters for the 200–500 m layer and \(r = 0.01\) and \(v = 100\) for the 500–4810 m layer. We also calculated a range of \(S\) for each combination of the parameters \(r\) and \(v\) assumed by Giering et al. (2017) (\(r = 0.01–0.5\); \(v = 10–200\)) for the 200–500 m layer. For the 500–4810 m layer, we used narrower ranges for \(r\) (0.01–0.1) and \(v\) (50–200) because the highest mineralization rate (0.5 d⁻¹) and the lowest sinking speed (10 m d⁻¹) were unrealistic for the deeper layer (Trull et al. 2008; Honda et al. 2009, 2013). The range of \(\Delta \text{POC}\) was then calculated using the minimum and maximum estimates of \(S\), which indicated the range of uncertainty associated with the parameter settings.

To compare PCD and the POC supply on a yearly basis, the time-series curves for the depth-integrated POC (for the 200–500 m and 500–4810 m layers) and POC fluxes (\(F_{200}, F_{500}, F_{4810}\)) were integrated over the sampling periods of 867 d and 881 d (between January 2010 and June 2012) for Sta. K2 and Sta. S1, respectively, and the integrated values were divided by 365 d. Sporadically high \(F_{4810}\) values observed at Sta. K2 were not included in the calculation (see “Results” section). These yearly POC fluxes (g C m⁻² yr⁻¹) were used to calculate the yearly POC supply; i.e., yearly \(\Delta \text{POC}_{200-500} = yearly \ F_{200} - yearly \ F_{500}\), and yearly \(\Delta \text{POC}_{500-4810} = yearly \ F_{500} - yearly \ F_{4810}\).

Results

Overview of oceanographic features and prokaryotic parameters

At Sta. K2, the euphotic-layer and mixed-layer depths varied within ranges of 49–95 m and 25–128 m, respectively (Table 1). The temperature profiles indicated the presence of dichothermal water (DTW) (temperature: 1.3–2.3°C; depth: 100 m) and mesothermal water (MTW) (temperature: 3.6–3.7°C; depth: 300 m) (Ueno and Yasuda 2000) (Fig. 2a). The upper boundaries of the North Pacific deep water (NPDW; \(\sigma_{th} = 27.69\)) and the lower circumpolar deep water (LCDW; \(\sigma_{th} = 27.77\)) (Kawabe and Fujio 2010; Wakita et al. 2010) were located at depths of 2000 m and 3500 m, respectively (Fig. 2b). The concentrations of DOC generally decreased with depth (range: 36–51 \(\mu\)mol L⁻¹) between the DTW and the upper boundary of the NPDW, but were relatively uniform within the NPDW and LCDW (Fig. 2b). Prokaryotic production and abundance decreased by 10³ and 10-fold, respectively, from the bottom of the euphotic layer to the bottom of the NPDW (depth: 3500 m) (Fig. 2c,d). In the LCDW, the depth-dependent changes in production and abundance
were moderate, except that they tended to increase with depth near the seafloor.

At Sta. S1, the euphotic-layer depth (88–112 m) exceeded the mixed-layer depth (15–64 m) throughout the study period (Table 1). The core and bottom of the North Pacific subtropical mode water (STMW) (following the definition of Hanawa and Talley 2001) were located at depths of 200 m and 300 m, respectively (Fig. 2e,f). Below the STMW were the North Pacific intermediate water (NPIW; core depth: 700 m), NPDW (upper boundary: 2200 m), and LCDW (upper boundary: 4000 m). The temporal variability in the DOC concentrations was relatively large in the STMW (200–300 m) (Fig. 2f). In this layer, the DOC concentrations were higher in spring (2011) and summer (2011, 2012) than in winter or fall (2010) (inset in Fig. 2f). Below 300 m, the DOC concentrations decreased sharply to the upper boundary of the NPDW (2200 m) and were relatively uniform within the NPDW and LCDW. Prokaryotic production and abundance decreased by 10³ and 10-fold, respectively, from the bottom of the euphotic layer to the upper boundary of the LCDW. Prokaryotic production and abundance changed only moderately with depth, except for a sharp increase near the seafloor.

Fig. 2. Vertical profiles of the hydrographic and biogeochemical parameters at Sta. K2 (upper column) and Sta. S1 (lower column). Salinity and water temperature (a, e), DOC concentration and potential density (σθ) (b, f), heterotrophic prokaryotic production (c, g), and prokaryotic abundance (d, h) are shown. DTW, dichothermal water; LCDW, lower circumpolar deep water; MTW, mesothermal water; NPDW, North Pacific deep water; NPIW, North Pacific intermediate water; STMW, subtropical mode water. Gray and black boxes indicate the euphotic layer and seafloor, respectively. Open circles: winter (2010); squares: fall (2010); diamonds: spring (2011); triangles: summer (2011); inverted triangles: summer (2012).
The cruises were conducted during different phases of the seasonal cycles in surface temperature and Chl \(a\) concentration (Fig. 3a,b,f,g). At Sta. K2, the Chl \(a\) concentration showed a recurrent seasonal cycle, with the seasonal maximum in summer (August–September) and minimum in winter (January–March). Consistent with this pattern, \(F_{200}\) also tended to be higher in summer than in winter (Fig. 3c). The phase of the flux change was evaluated from general seasonal patterns of \(F_{200}\) and \(F_{500}\) (Fig. 3c,d). The October 2010 (MR10-06) cruise was conducted during the declining phase of \(F_{200}\), whereas the cruises in April 2011 (MR11-03) and June 2011 (MR11-05) were conducted during the increasing phase of \(F_{200}\) (Fig. 3c; Table 2). Similarly, \(F_{500}\) tended to decline from the summer (July) of 2010 to the winter (February) of 2011, followed by an increase in \(F_{500}\) toward the summer (July) of 2011 (Fig. 3d; Table 2). The seasonal pattern was less evident for \(F_{4810}\) with sporadically high values in May 2010 and September and October 2011 (Fig. 3e).

At Sta. S1, the Chl \(a\) concentrations were generally high in spring (April–May) and low in summer (July–September) (Fig. 3g). \(F_{200}\) varied greatly (range: 1.39–139) during our

**Fig. 3.** Temporal variabilities in sea surface temperature (SST) (a, f) and surface Chl \(a\) concentration (b, g), and sinking POC fluxes determined at depths of 200 m \((F_{200})\) (c, h), 500 m \((F_{500})\) (d, i), and 4810 m \((F_{4810})\) (e, j) at Sta. K2 (left column) and Sta. S1 (right column). Solid lines are three-point running averages, after the exclusion of anomalously high values (open cycles). Gray shading indicates the cruise periods (see Table 1 for details).
Table 2. Sinking POC flux (mg C m\(^{-2}\) d\(^{-1}\)) at depths of 200 m (F\(_{200}\)), 500 m (F\(_{500}\)), and 4810 m (F\(_{4810}\)), the parameters (S, mg C m\(^{-2}\) d\(^{-1}\)) for Giering’s correction of the flux attenuation in the 200–500 m layer (S\(_{200–500}\)) and 500–4810 m layer (S\(_{500–4810}\)), and the POC supply (flux attenuation; mg C m\(^{-2}\) d\(^{-1}\)) for the 200–500 m layer (ΔPOC\(_{200–500}\)) and 500–4810 m layer (ΔPOC\(_{500–4810}\)). \(\alpha\), the slope of the linear increase or decrease in the sinking POC flux over time (see text for details).

| Station | Month/Year (Cruise) | F\(_{200}\) | F\(_{500}\) | F\(_{4810}\) | S\(_{200–500}\)^* | S\(_{500–4810}\)^* | ΔPOC\(_{200–500}\)^* | ΔPOC\(_{500–4810}\)^* | Remarks |
|---------|---------------------|------------|------------|------------|---------------|----------------|----------------|----------------|---------|
| Sta. K2 | January 2010 (MR10-01) | 8.8 | 4.2 | 2.1 | −1.5 | −4.4 | 6.1 | 6.5 | Assumed to be a declining phase. For the derivation of S, \(\alpha\) determined for October 2010 was used (−0.31 for F\(_{200}\) and −0.13 for F\(_{500}\)). F\(_{200}\), F\(_{500}\), and F\(_{4810}\) are the average of the data obtained between February and March. Declining phase; \(\alpha\) was −0.31 for F\(_{200}\) for the period 08/21/2010–01/12/2011 (\(r^2=0.70, p<0.05, n=6\)) and −0.13 for F\(_{500}\) for the period 07/28/2010–12/19/2010 (\(r^2=0.86, p<0.001, n=11\)). F\(_{200}\) and F\(_{500}\) are based on the linear regression models. F\(_{4810}\) is the average of the data obtained in November. |
|          | October 2010 (MR10-06) | 21.5 | 9.5 | 2.7 | −1.5 | −4.4 | 13.5 | 11.2 | Increasing phase. \(\alpha\) was 0.12 for F\(_{200}\) for the period 12/31/2010–07/21/2011 (\(r^2=0.92, p<0.05, n=4\)) and 0.05 for F\(_{500}\) for the period 12/31/2010–07/21/2011 (\(r^2=0.36, p<0.05, n=14\)). F\(_{200}\) and F\(_{500}\) are based on the linear regression models. F\(_{4810}\) is the average of the data obtained in April. The negative value of ΔPOC\(_{500–4810}\) indicates that F\(_{4810}\) exceeded F\(_{500}\). |
|          | April 2011 (MR11-03)  | 17.0 | 4.4 | 1.6 | 0.6 | 1.9 | 12.0 | 0.9 | Increasing phase; \(\alpha\) was 0.12 for F\(_{200}\) for the period 12/31/2010–07/21/2011 (\(r^2=0.92, p<0.05, n=4\)) and 0.05 for F\(_{500}\) for the period 12/31/2010–07/21/2011 (\(r^2=0.36, p<0.05, n=14\)). F\(_{200}\) and F\(_{500}\) are based on the linear regression models. F\(_{4810}\) is the average of the data obtained between June and July. |
|          | June 2011 (MR11-05)   | 25.9 | 8.1 | 4.2 | 0.6 | 1.9 | 17.2 | 2.1 | Assumed to be an increasing phase. For the derivation of S, \(\alpha\) determined for June 2011 was used (0.12 for F\(_{200}\) and 0.05 for F\(_{500}\)). F\(_{200}\) is the datum obtained on 05 June 2012. F\(_{500}\) is the average of the data obtained between April and May. F\(_{4810}\) is the average of the data obtained in May. |
|          | June 2012 (MR12-02)   | 13.9 | 9.6 | 5.0 | 0.6 | 1.9 | 3.7 | 2.8 | F\(_{200}\) and F\(_{500}\) are the average of the data obtained between 22 February and 06 March. F\(_{4810}\) is the average of the data obtained between February and March. |
| Sta. S1  | January 2010 (MR10-01) | 23.1 | 10.0 | 4.1 | N/A | N/A | 13.1 | 5.8 | F\(_{200}\) is the average of the data obtained between 21 August and 07 December. F\(_{500}\) is the average of the data obtained between 28 July and 19 December. F\(_{4810}\) is the average of the data obtained between October and November. |
|          | November 2010 (MR10-06) | 5.1 | 1.9 | 1.1 | N/A | N/A | 3.2 | 0.8 | Declining phase for F\(_{500}\); \(\alpha\) was −0.04 for the period 03/25/2011–08/31/2011 (\(r^2=0.52, p<0.05, n=10\)). F\(_{200}\) is the average of the data obtained between 25 March and 24 May. F\(_{500}\) is based on the linear regression model. F\(_{4810}\) is the average of the data obtained in April. Negative values of ΔPOC\(_{500–4810}\) indicate that F\(_{4810}\) exceeded F\(_{500}\). |
|          | April 2011 (MR11-03)  | 8.9 | 4.7 | 6.1 | N/A | −1.3 | 4.2 | −0.14 | Declining phase for F\(_{500}\); \(\alpha\) was −0.04 for the period 03/25/2011–08/31/2011 (\(r^2=0.52, p<0.05, n=10\)). F\(_{200}\) and F\(_{4810}\) are the average of the data obtained between June and July. F\(_{500}\) is based on the linear regression model. |
|          | July 2011 (MR11-05)   | 6.7 | 1.6 | 1.1 | N/A | −1.3 | 5.1 | 1.7 | Declining phase; \(\alpha\) was −0.27 for F\(_{200}\) for the period 03/10/2012–06/14/2012 (\(r^2=0.70, p<0.01, n=8\)) and −0.07 for F\(_{500}\) for the period 03/10/2012–06/14/2012 (\(r^2=0.78, p<0.01, n=7\)). F\(_{200}\) and F\(_{500}\) are the average of the data obtained between May and June. F\(_{4810}\) is the datum obtained on 10 March 2012. |
|          | June 2012 (MR12-02)   | 11.5 | 1.4 | 0.29 | −1.3 | −2.6 | 11.3 | 3.7 | |

N/A, not applicable.
* Ranges in parentheses indicate the uncertainty associated with the parameter settings for the estimation of S (see text).
investigation, although its seasonality and link to the variability in Chl$\alpha$ were less clear (Fig. 3h). This made it difficult to assign the phase (i.e., either declining or increasing) of $F_{200}$ on each water-sampling occasion, except that the June 2012 (MR12-02) sampling was possibly conducted during the declining phase of $F_{200}$ (Fig. 3h; Table 2). $F_{500}$ and $F_{4810}$ tended to be high in winter, and the peaks generally corresponded to periods of high Chl$\alpha$ concentrations (Fig. 3i,j). April 2011 (MR11-03), July 2011 (MR11-05), and June 2012 (MR12-02) were defined as declining phases of $F_{500}$ (Fig. 3i; Table 2), whereas other occasions were neither declining nor increasing phases (Table 2).

**Prokaryote production, biomass, and turnover time**

The depth-integrated prokaryotic production (PP), prokaryotic biomass (PB), and turnover time ($T = PB/PP$) were calculated for the 200–500 m layer ($PP_{200-500}$, $PB_{200-500}$, and $T_{200-500}$, respectively) and the 500–4810 m layer ($PP_{500-4810}$, $PB_{500-4810}$, and $T_{500-4810}$, respectively) (Fig. 4). $PP_{200-500}$ varied more widely at Sta. S1 (range: 0.48–3.6 mg C m$^{-2}$ d$^{-1}$) than at Sta. K2 (range: 0.84–1.9 mg C m$^{-2}$ d$^{-1}$) (Fig. 4a,b). At Sta. S1, $PP_{200-500}$ tended to be higher in spring (April 2011) and summer (July 2011 and June 2012) than in winter (January 2010) and fall (November 2010). This tendency is consistent with the seasonal change in the DOC concentration in the upper mesopelagic layer at Sta. S1 (Fig. 2f). The seasonal variability in $PB_{200-500}$ was less pronounced at both stations (Sta. K2, range: 0.64–0.75 g C m$^{-2}$; Sta. S1, range: 0.43–0.68 g C m$^{-2}$) (Fig. 4a,b). $T_{200-500}$ varied in the range of 0.93–2.2 yr at Sta. K2 and 0.51–2.5 yr at Sta. S1 (Fig. 4a,b).

The seasonal variabilities in $PP_{500-4810}$ and $PB_{500-4810}$ were small at both Sta. K2 and Sta. S1 (Fig. 4c,d). The mean $PP_{500-4810}$ at Sta. K2 ($1.6 \pm 0.38$ mg C m$^{-2}$ d$^{-1}$) was 2.4-fold larger than the corresponding value at Sta. S1 ($0.68 \pm 0.13$ mg C m$^{-2}$ d$^{-1}$) (the difference in the mean values was significant; $p < 0.001$, Student’s $t$-test). Similarly, the mean PB was 2.0-fold larger at Sta. K2 ($2.5 \pm 0.25$ g C m$^{-2}$) than at Sta. S1 ($1.2 \pm 0.047$ g C m$^{-2}$) ($p < 0.001$, Student’s $t$-test). $T_{500-4810}$ varied in the range of 3.2–5.9 yr at Sta. K2 and 4.0–6.5 yr at Sta. S1.

**Organic carbon supply and consumption**

The non-steady-state effect involved in the estimation of the POC flux attenuation (Giering et al. 2017) was corrected
in the calculation of $\Delta$POC$_{200-500}$ and $\Delta$POC$_{500-4810}$ from the data collected during either the declining or increasing phase of $F_{200}$ (for $\Delta$POC$_{200-500}$) and $F_{500}$ (for $\Delta$POC$_{500-4810}$). For this calculation, the linear regression slopes of $F_{200}$ or $F_{500}$ were derived for each phase (Table 2), except for some cases at Sta. K2 ($F_{200}$ and $F_{500}$ for January 2010 and June 2012) for which the data were insufficient for a regression analysis. In these cases, the slope obtained for another period of the corresponding phase was used for the correction (Table 2). Depending on the phase, Giering’s correction leads to either an upward (for a declining phase) or downward (for an increasing phase) revision of $\Delta$POC (Table 2; Fig. 5). With the default parameter settings (see “Materials and methods” section), the extent of the revision was minor for $\Delta$POC$_{200-500}$ (the ratio of corrected to uncorrected values was 0.86–1.32) (Fig. 5a,b), whereas it was relatively large for $\Delta$POC$_{500-4810}$ by 1.9-fold at Sta. K2 and by 1.7-fold at Sta. S1 (Fig. 5c,d).

On a yearly basis, the POC supply was sufficient to fulfill the PCD in the 200–500 m layer; PCD$_{200-500}$ accounted for 60% of $\Delta$POC$_{200-500}$ at Sta. K2 and 58% at Sta. S1 (Fig. 6a). In the 500–4810 m layer, PCD$_{500-4810}$ exceeded $\Delta$POC$_{500-4810}$ by 1.9-fold at Sta. K2 and by 1.7-fold at Sta. S1 (Fig. 6b).

**Discussion**

**Temporal variability in POC flux and prokaryotic parameters**

We compared the time-series POC flux data with the seasonally determined prokaryotic biomass, production, and PCD in the deep ocean. Although the prokaryotic data were
collected much less frequently (five times) than the POC flux data, they still represented different phases in the seasonal cycle of POC flux. Importantly, the degree of the temporal variability in the prokaryotic biomass and production was much smaller than that in the POC flux. In fact, at both Sta. K2 and Sta. S1, $F_{200}$ varied over a few orders of magnitude (Fig. 3), whereas prokaryotic production and biomass varied within a rather limited range, especially in the 500–4810 m layer (less than twofold difference; Fig. 4c,d). The prokaryotic responses to seasonal pulses in the organic carbon supply were probably smoothed and attenuated at depth because the prokaryotic turnover times were slow. Our estimated turnover times of 0.5–2.5 yr for the 200–500 m layer and 3.2–6.5 yr for the 500–4810 m layer suggest that prokaryotic parameters are only weakly coupled to the POC flux on the seasonal timescale. Similar turnover times of prokaryotes (from months to years) have been reported in the meso- and bathypelagic water columns of the central Pacific Ocean (Nagata et al. 2000; Yokokawa et al. 2013) and the western Arctic Ocean (Uchimiya et al. 2013). However, the turnover times of prokaryotes determined from prokaryote production and biomass are the mean turnover times for the bulk community. It is possible that the bulk prokaryote community consists of rapidly growing subpopulations and more slowly growing (or even dormant or dead) subpopulations (del Giorgio and Gasol 2008). Interactions between microbes and organic matter occur in the micron-sized space surrounding individual microbial cells on a time scale of minutes to hours. Although microscale processes are thought to have ramifications for basin-scale carbon mineralization in the deep ocean over years, meshing such vast time scales (from minutes to years) and spatial scales (from micrometers to hundreds of kilometers) remains a major challenge in microbial ecology (Kirchman 2012). In the present study, when we took a macroscopic approach (Brown 1995), we found that the prokaryote biomass and production in the 500–4810 m layer were systematically (about twofold) greater at Sta. K2 than at Sta. S1 (Fig. 4c,d). This was consistent with the finding that the integrated $\Delta{\text{POC}}_{500-4810}$ was about twofold greater at Sta. K2 than at Sta. S1 (Fig. 6b). Therefore, there is an apparent coupling between the organic carbon supply and the prokaryotic biomass and production on large time scales (integrated over 2.5 yr) and spatial scales (across subarctic and subtropical regions of the western North Pacific). Previous studies using snapshot data have also found that prokaryotic biomass and production usually correlated positively with the sinking POC fluxes in bathypelagic waters over a large geographic scale (Nagata et al. 2000; Yokokawa et al. 2013). An important implication of these findings is that the prokaryote biomass and production in deep water do not necessarily respond to the seasonal variability in the POC flux, but do reflect the POC flux integrated over a longer timescale.

**Balancing organic carbon supply and consumption**

The $\Delta{\text{POC}}$ and PCD must be compared with great caution because methodological problems are involved. There are uncertainties in the estimation of PCD, as discussed by Burd et al. (2010), because of the inherent errors associated with the conversion of $^3$H-leucine incorporation to prokaryote production and the errors associated with the assumption of gross growth efficiencies. In the present study, PCD was estimated as the average result obtained with two different assumptions (Methods 1 and 2), and the range of the estimates was treated as an error. These errors were generally moderate, being predominantly $<7\%$ for the PCD estimates. Another source of error is associated with the measurement of prokaryote production using decompressed samples. Decompression may result in the under- or overestimation of prokaryotic production, although the extent (and even direction) of this error is poorly understood (Nagata et al. 2010; Tamburini et al. 2013). Uncertainties are also involved in the estimation of the carbon supply ($\Delta{\text{POC}}$), which is calculated from the sinking POC flux data. This uncertainty can arise from several sources, including variable particle collection efficiencies (hydrodynamic effects), the presence of zooplankton swimmers, and particle solubilization in the trap (Buesseler et al. 2007; Burd et al. 2010). Spatial and temporal heterogeneity in the sinking POC flux and the lateral transport of particles may also introduce errors into the estimation of $\Delta{\text{POC}}$ (Neuer et al. 1997; Alonso-Gonzále et al. 2009; Giering et al. 2017), although the effect of lateral transport is probably small at Sta. K2 and Sta. S1 (Honda et al. 2009, 2013). In the present study, the effects of the
temporal variability in the POC flux on the estimation of ΔPOC were corrected, whenever possible, using a model proposed by Giering et al. (2017). This correction either increased or reduced the ΔPOC estimates, and the size of the correction was up to fourfold in the 500–4810 m layer (Fig. 5), although this depended on the assumptions made for the parameters used in the model (v and r).

At both Sta. K2 and Sta. S1, the carbon supply (ΔPOC) usually exceeded PCD in the 200–500 m layer, and even when ΔPOC was less than PCD the difference between the two estimates was generally moderate. A notable exception occurred at Sta. S1 in April 2011, when PCD was fourfold higher than ΔPOC. On this sampling occasion, there was an STMW intrusion that brought DOC into the upper mesopelagic layer (Fig. 2f), suggesting that advectively transported DOC was available for prokaryote consumption. The data from that individual cruise showed that the sinking POC supply was generally sufficient to support PCD in the 200–500 m layer, consistent with the results when ΔPOC and PCD were compared on a yearly basis. From the yearly integrated data, the percentage of PCD relative to ΔPOC was 60% at Sta. K2 and 58% at Sta. S1 (Fig. 6). These results were consistent with the previous estimate made in the North Atlantic (Giering et al. 2014), where a sufficient amount of organic carbon was supplied by sinking POC flux to fulfill PCD in the mesopelagic layer during summer. The ΔPOC not respired by prokaryotes is available for consumption by mesopelagic zooplankton (Giering et al. 2014).

In the 500–4810 m layer, PCD generally exceeded ΔPOC (without Giering’s correction), and was up to 5–6-fold greater than ΔPOC in January 2010 at Sta. K2 and in July 2011 at Sta. S1. The deviation was also large in April 2011 at Sta. S1, when ΔPOC300–4810 was undetectably low. With correction for the non-steady-state effect of sinking POC (Giering et al. 2017), the deviation was attenuated in January 2010 (Sta. K2) and July 2011 (Sta. S1), whereas it was enhanced from 2.5-fold to 7.3-fold in April 2011 at Sta. K2 (Fig. S5). Therefore, from the individual data collected during each cruise, PCD could largely exceed ΔPOC, even after Giering’s correction. One possible explanation of this excess PCD relative to ΔPOC is that the prokaryotes were fueled by the organic carbon that was delivered into the deep layer during the period preceding the occasion of water sampling. Semilabile DOC (Follett et al. 2014) and neutrally buoyant particles, including transparent exopolymer particles (Bochdansky et al. 2010; Yamada et al. 2017), released from the sinking POC into the surrounding seawater might be slowly (e.g., over months) consumed by the prokaryotes in the water column during the subsequent period. This temporal uncoupling between the supply (by the sinking POC) and consumption (by the prokaryotes) of organic carbon could result in excess PCD relative to ΔPOC, depending on the time of the observation. This model is consistent with the slow turnover time of prokaryotes in the deeper layer, as discussed above. The results of our comparison of ΔPOC and PCD on a yearly basis (Fig. 6), which showed that PCD was equivalent to ΔPOC within a factor of 2, also support this model. This deviation is much smaller than the deviation reported previously in the bathypelagic layer based on snapshot data (Reinthaler et al. 2006; Uchimiya et al. 2013).

Despite the diminished carbon imbalance, our data still indicate that the POC flux was insufficient to meet the PCD in the deeper layer. Organic particles that were not captured by the sediment traps (Baltar et al. 2009; Close et al. 2013) and autochthonous carbon fixation by chemosynthesis (Hansman et al. 2009; Reinthaler et al. 2010) might supply organic carbon for heterotrophic microbial consumption at depth. Taken together, our comparison of the supply and consumption of organic carbon in deep water indicates that the enigma of the high PCD relative to the POC supply is partly resolved by a temporal uncoupling between supply and consumption. Complete reconciliation of the imbalance in the deep ocean carbon budget requires further scrutiny, focusing on the mechanisms of the temporal uncoupling between organic carbon delivery and consumption, and the missing sources of organic carbon in deep water.

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Acknowledgments
We thank the captain and crew of the R/V Mirai and the staff of the Global Ocean Development and Marine Works Japan for their support during the cruises. K. Sasaoka provided the satellite data, K. Hamasaki helped to develop the observation plan, and R. Kaneko supported the sample collection. T. Fujiyoshi provided valuable information regarding the biogeochemical parameters. We are deeply grateful to the editor and anonymous reviewers for their productive comments on our manuscript. This study was supported by JSPS KAKENHI grants (24241003 and 15H01725) awarded to TN and HF, and by a JSPS Grant-in-Aid for Scientific Research awarded to MU. This paper is dedicated to the memory of Toshiro Saino.

Conflict of Interest
None declared.

Submitted 21 January 2017
Revised 30 December 2017
Accepted 15 March 2018

Associate editor: Thomas Anderson