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Variability in photosynthetic production of dissolved and particulate organic carbon in the North Pacific Subtropical Gyre

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The partitioning of photosynthetically-derived organic carbon between particulate and dissolved phases has important implications for marine carbon cycling. In this study we utilized ^{14}C-bicarbonate assimilation to quantify rates of photosynthetic production of both particulate and dissolved organic carbon (DOC) at Station ALOHA (22°45′N, 158°W) in the North Pacific Subtropical Gyre (NPSG). At near-monthly time scales over ∼5 years, we examined retention of ^{14}C-labeled organic matter by both glass fiber filters and 0.2 μm pore size polycarbonate membrane filters that are commonly used for measurements of ^{14}C-based plankton productivity. Use of polycarbonate filters resulted in significantly lower (averaging 60%) estimates of ^{14}C-production compared to glass fiber filters. Coincident measurements of chlorophyll *a* concentrations from both 0.2 μm polycarbonate and glass fiber filters were not significantly different, suggesting the differences in ^{14}C-productivity between these filter types did not derive from differences in retention of photosynthetic biomass by these filters. Moreover, consistent with previous studies, results from experiments aimed at quantifying retention of organic matter by these filters suggested differences resulted from retention of DOC by glass fiber filters. We also quantified rates of ^{14}C-DOC production to evaluate the partitioning of photosynthetic production between dissolved and particulate phases over daily to monthly time scales in this ecosystem. Unlike the strong depth dependence observed in measurements of particulate organic carbon production, measured rates of ^{14}C-DOC demonstrated no clear depth dependence. On average, depth-integrated (0–75 m) rates of ^{14}C-DOC production rates were equivalent to 18 ± 10% of the total (particulate and dissolved) productivity. Our findings indicate that in this oligotrophic ecosystem, rates of dissolved and particulate production can be temporally decoupled over daily to monthly time scales.

Keywords: primary productivity, dissolved organic carbon, North Pacific Ocean, Station ALOHA

Introduction

Oceanic net primary production accounts for approximately 50 Pg C yr^{-1}, and much of this productivity occurs in the vast, low nutrient subtropical ocean gyres (Behrenfeld and Falkowski, 1997; Field et al., 1998). Dissolved organic carbon (DOC), operationally defined as reduced carbon substrates passing through filters (typical pore sizes ranging 0.2–0.7 μm), constitutes >90% of total marine organic carbon inventories (Druffel et al., 1992; Hedges, 1992; Kaiser and Benner, 2009). Despite low inorganic nutrient concentrations throughout the upper euphotic zone of the subtropical gyres, concentrations of DOC are enriched in these ecosystems (Hansell et al., 2009). Hence, quantification of rates of DOC production and subsequent utilization are central to constraining carbon cycling in these systems.

A suite of processes can result in DOC production. These include direct release of organic material from phytoplankton cells either passively (Bjørnsen, 1988) or actively (Fogg, 1966; Lignell, 1990; Marañón et al., 2004). High light (Hellebust, 1965; Cherrier et al., 2014) and nutrient limitation (Lancelot, 1983; Conan et al., 2007; López-Sandoval et al., 2011) may also promote phytoplankton DOC release. Processes such as viral lysis and inefficient predation can also constitute major DOC production pathways (Lampert, 1978; Banse, 1995; Hygum et al., 1997; Wilhelm and Suttle, 1999; Møller et al., 2003; Møller, 2005; Suttle, 2005; Evans et al., 2009; Saba et al., 2011). Rates of DOC production are often measured by tracing phytoplankton assimilation of radiolabeled (^{14}C) inorganic carbon and quantifying the subsequent accumulation of ^{14}C-labeled DOC in seawater (Schindler et al., 1972; Baines and Pace, 1991; Carlson, 2002).

Measurement of ^{14}C bicarbonate assimilation into autotrophic biomass (Steemann Nielsen, 1952) has proven a sensitive method for estimating primary productivity rates in aquatic ecosystems and is often reported as approximating net primary productivity (Peterson, 1980; Bender et al., 1987; Marra, 2009; Pei and Laws, 2013). However, studies utilizing this methodology often do not quantify rates of DOC production (hereafter termed ^{14}C-DOC), or estimate respiratory losses during the incubation period; consequently, organic carbon production may be underestimated by this approach. Direct ^{14}C-DOC measurements have been made in diverse aquatic ecosystems (Baines and Pace, 1991), with rates in the open oceans typically ranging between 10 and 40% of particulate carbon production (Karl et al., 1998; Carlson et al., 2000; Morán and Estrada, 2001; Teira et al., 2001, 2003; Marañón et al., 2004; Conan et al., 2007).

The choice of filters utilized for measurements of particulate carbon production is an important consideration. Several studies have compared the retention of organic matter by various types of filters commonly used in aquatic systems (Maske and Garcia-Mendoza, 1994; Chavez et al., 1995; Karl et al., 1998; Morán et al., 1999). Glass fiber filters commonly used for these measurements are known to retain both ^{14}C-DOC and ^{14}C-labeled particulate carbon (Karl et al., 1998). However, to date, there are relatively few reports describing how different filter types influence derived rates of ^{14}C-productivity in the open ocean. In a comparative study of filter retention characteristics, Morán et al. (1999) reported greater retention of ^{14}C-labeled organic material on glass fiber filters compared to polycarbonate filters. However, the study also observed differences in the retention efficiencies of these filters in different ecosystems, suggesting the structure of planktonic communities and the relative importance of DOC to total organic matter productivity by these communities influences the retention characteristics of these filters (Morán et al., 1999).

The North Pacific Subtropical Gyre (NPSG) is one of the largest open ocean habitats on the planet. Since 1988, the Hawaii Ocean Time-series (HOT) program has sustained near-monthly shipboard measurements at Station ALOHA (A Long-Term Oligotrophic Habitat Assessment; 22°45′N, 158°W) in the NPSG, where oligotrophic upper ocean waters exhibit seasonality in various biogeochemical processes and properties (Campbell et al., 1994; Winn et al., 1995; Karl et al., 2001; Landry et al., 2001; Letelier et al., 2004; Dore et al., 2008; Church et al., 2009). A number of previous studies indicate that rates of primary production at Station ALOHA demonstrate moderate seasonality, with rates higher in summer and lower in winter (Karl et al., 1996; Letelier et al., 1996; Quay et al., 2010; Church et al., 2013). A previous study quantifying ^{14}C-DOC production rates at Station ALOHA revealed that ^{14}C-DOC comprised a relatively large fraction (14–51%) of daily photosynthetic production (Karl et al., 1998). However, there is limited information on temporal variability associated with the partitioning of organic carbon production into dissolved and particulate phases in this ecosystem.

In the present study, we assess the magnitude and partitioning of primary production between particulate and dissolved pools at Station ALOHA. We evaluate retention characteristics of glass fiber and polycarbonate filters commonly used for measurements of ^{14}C-based productivity and concentrations of chlorophyll *a*. Our results confirm that rates of ^{14}C productivity were significantly greater when derived using glass fiber filters compared to polycarbonate filters, despite no significant differences in the retention of chlorophyll *a* by these filters. We also examined rates of ^{14}C-DOC production to test the hypothesis that rates of ^{14}C-DOC production would demonstrate similar time-varying patterns as rates of ^{14}C-particulate carbon production. However, despite periods of moderate seasonality in photosynthetic production of particulate carbon, ^{14}C-DOC production was more temporally variable than coincident rates of ^{14}C-particulate carbon production.

Materials and Methods

Chlorophyll *a* and ^{14}C-based Productivity Measurements

Sampling for this study was conducted at near-monthly time scales at Station ALOHA on HOT program cruises during two separate periods, October 2004 to October 2007 and April 2010 to October 2012. During the initial period of the study (2004–2007), we compared retention characteristics of 25 mm diameter 0.2 μm

pore size polycarbonate membrane filters (Millipore) and 25 mm diameter glass fiber filters (Whatman GF/F) for subsequent analyses of both chlorophyll *a* concentrations and rates of ^{14}C-productivity. To compare retention of chlorophyll *a* by these filter types, paired seawater samples were collected from pre-dawn hydrocasts using a conductivity-temperature-density (CTD) rosette sampler equipped with 12 L polyvinyl chloride bottles. Seawater from six discrete depths (5, 25, 45, 75, 100, and 125 m) was sampled from the CTD rosette bottles into 150 ml amber polyethylene bottles. The entire 150 ml sample was filtered onto either polycarbonate or glass fiber filters. Filters were immersed in 5 ml of 100% HPLC grade acetone in 7 ml glass culture tubes and placed at –20°C to passively extract. After 7 days (Letelier et al., 1996), tubes were removed from the freezer, filters were removed, and extracted chlorophyll in the acetone was quantified using a Turner Designs Model 10-AU fluorometer (Strickland and Parsons, 1972).

Sampling for ^{14}C-based measurements of particulate production occurred on near-monthly HOT program cruises throughout the study period. We measured rates of ^{14}C-assimilation into particulate carbon using polycarbonate filters to harvest plankton biomass (hereafter ^{14}C-PC), and compared these rates to coincident (in both time and depth) core HOT program measurements of ^{14}C-assimilation into plankton biomass (Letelier et al., 1996), based on use of glass fiber filters (hereafter ^{14}C-GFF). During the latter period of observations (2010–2013) we also measured ^{14}C-DOC production rates. Seawater for the ^{14}C-based productivity measurements was collected from the same predawn CTD hydrocasts sampled for chlorophyll *a* concentrations. Water for the productivity measurements was sampled from the CTD rosette bottles into acid-cleaned 500-ml polycarbonate bottles. A total of four replicate 500 ml bottles were subsampled per depth and each bottle was spiked with ∼1.85 MBq ^{14}C-bicarbonate. One hundred milliliters from one replicate per depth was immediately vacuum filtered through a polycarbonate filter; these "time zero" filtrates provided a ^{14}C-DOC blank and provided information on background adsorption of inorganic ^{14}C to the filters. Time zero filters were placed in 20 ml glass scintillation vials (Kimble Chase) and stored at −20°C until shore-based laboratory processing. The remaining three bottles were hung on a free-drifting array, deployed before dawn, and incubated at their initial collection depths throughout the photoperiod (typically 11–13 h). After sunset the array was recovered, and 100 ml subsamples of all bottles were filtered under gentle vacuum (<50 mm Hg) onto polycarbonate filters that were then placed in scintillation vials and frozen (−20°C). The total radioactivity added to each sample bottle was determined by subsampling 250 µl aliquots into scintillation vials containing 500 µl of β-phenylethylamine. At the shore-based laboratory, filters were acidified by the addition of 1 ml of 2 M hydrochloric acid (HCl) and allowed to passively vent (uncapped) for ∼24 h to remove inorganic ^{14}C. Ten milliliters of Ultima Gold LLT liquid scintillation cocktail was added to all vials (acidified filters and vials for determining total radioactivity) and the resulting radioactivity was determined using a Perkin Elmer 2600 liquid scintillation counter; glass fiber filters were recounted after 30 days (Karl et al., 1998).

Measurements of ^{14}C-DOC Production

Over a ∼2.5-year period (April 2010–October 2012), we measured ^{14}C-DOC production from the same vertical profiles used for determination of ^{14}C-PC production by utilizing filtrates derived from ^{14}C-PC rate measurements. These 0.2 µm filtrates were collected from both time zero samples and triplicate bottles incubated *in situ* on the free-drifting array into 125 ml polyethylene amber bottles and stored frozen (−20°C) until subsequent processing for determination of ^{14}C-DOC productivity. In the shore-based laboratory, these samples were processed following the ^{14}C-DOC methodology described in Karl et al. (1998). Briefly, 100 ml of the ^{14}C-PC filtrates were thawed, poured into 500 ml polyethylene separatory funnels, and acidified by the addition of 500 µl of 2 M sulfuric acid (H_2SO_4). Samples were vigorously bubbled with air in a fume hood to remove $^{14}CO_2$. After at least 6 h of bubbling, a 70 ml subsample was removed from each separatory funnel and poured into a 100 ml glass serum bottle containing 1 ml of 2 M sodium hydroxide (NaOH) and 10 ml of 0.37 M potassium persulfate ($K_2S_2O_8$) in 1 M NaOH. Bottles were sealed with rubber stoppers, crimp sealed with an aluminum cap, and autoclaved at 126°C for 200 min; oxidizing ^{14}C-DOC to ^{14}C-labeled dissolved inorganic carbon (^{14}C-DIC) in an alkaline solution. Once cooled to room temperature, samples were uncapped and resealed using rubber sleeve stoppers holding plastic center wells containing ∼2 × 2 cm pieces of fluted chromatographic filter paper (Whatman 2) soaked with 0.2 ml of β-phenylethylamine. A syringe was used to inject 4 ml of 9 N H_2SO_4 into the solution, converting the ^{14}C-DIC to $^{14}CO_2$. Samples were stored undisturbed at room temperature, passively trapping the $^{14}CO_2$ on the β-phenylethylamine soaked wick. After 5 days, rubber sleeve stoppers were removed and center wells and wicks were placed in scintillation vials, followed by the addition of 10 ml of Ultima Gold LLT scintillation cocktail. Samples were subsequently counted on a Perkin Elmer Tri-Carb 2800TR liquid scintillation counter. Rates of ^{14}C-DOC production were computed for each cruise as the mean of the triplicate bottles from each depth minus the average ^{14}C-activity of the time zero (blank) samples. We defined a limit of detection for the ^{14}C-DOC analyses per cruise as the value of the mean time zero "blank" samples for that cruise plus three times the standard deviation of those time zero "blank" samples (Skoog and Leary, 1992). Measurements falling below the detection limit were assigned a value of zero for subsequent analyses, including calculation of mean rates.

Diel Variability in Productivity

On three separate occasions (April 2013, May 2013, and June 2013), we conducted experiments to examine short-term (diel) variability in production and loss of ^{14}C-PC, ^{14}C-GFF, and ^{14}C-DOC. Triplicate 500 ml samples were collected from 25 m depth from pre-dawn CTD hydrocasts, inoculated with ∼1.85 MBq ^{14}C-bicarbonate, and placed in a surface seawater cooled incubator shaded to 50% incident irradiance. Samples were incubated for varying lengths of time: predawn until noon

(∼6 h), full photoperiod (predawn to dusk, ∼12 h), or over a full day (predawn to predawn, ∼24 h). Following incubation, samples were filtered and processed as previously described for determination of ^{14}C-PC, ^{14}C-GFF, and ^{14}C-DOC. Rates of ^{14}C-DOC below the limit of detection were assigned a value of zero for these analyses.

Filter Retention Characteristics

We conducted an experiment designed to specifically evaluate retention characteristics of glass fiber and polycarbonate filters for measurements of ^{14}C-productivity and chlorophyll *a* (October 2014). For this experiment, 500 ml seawater samples were collected from the near-surface (25 m) ocean, inoculated with ∼1.85 MBq ^{14}C-bicarbonate and incubated from dawn to dusk in temperature controlled, shaded (50% incident irradiance) incubators. After sunset, triplicate 100 ml subsamples were vacuum filtered onto both polycarbonate and glass fiber filters individually, and onto these filters in series (i.e., glass fiber filters underlain by polycarbonate filter or polycarbonate underlain by glass fiber filter). Filters were placed in scintillation vials, acidified, and processed as previously described for determination of ^{14}C activities. From the same water sample, we also compared retention of planktonic chlorophyll *a* by glass fiber and polycarbonate filters; 125 ml was collected and filtered onto either a glass fiber filter, a polycarbonate filter, or onto these filters in series (as above). Chlorophyll concentrations were determined via fluorometric analysis as previously described.

An additional experiment (October 2014) was conducted to evaluate trapping of ^{14}C-organic carbon by glass fiber filters. Replicate 500 ml polycarbonate bottles containing whole seawater from Station ALOHA were inoculated with ∼1.85 MBq ^{14}C-bicarbonate and incubated in a temperature controlled and shaded incubator for the duration of the photoperiod. After dusk, incubations were terminated by filtering the sample bottles onto polycarbonate filters. The resulting filtrates were collected and triplicate 100 ml volumes were sequentially filtered onto new glass fiber filters, resulting in a filtrate that had been filtered a total of five separate times through five different glass fiber filters. In addition, triplicate 500 ml samples of the original 0.2 μm filtrate were filtered onto glass fiber filters to evaluate possible volume-dependent differences in the trapping of ^{14}C-DOC by these filters. The resulting ^{14}C activities associated with each glass fiber filter were determined as previously described.

We also evaluated the effects of filtering different volumes of the ^{14}C-productivity samples onto glass fiber and polycarbonate filters. For these comparisons, we examined paired primary production samples collected from the upper ocean (<45 m) at Station ALOHA, where 100 ml of seawater was filtered onto a polycarbonate filter, while varying volumes of seawater (100, 150, 400, or 500 ml) were filtered onto glass fiber filters. These comparative measurements were used to calculate the difference between the derived rates of ^{14}C-productivity on the glass fiber and polycarbonate filters (^{14}C-delta = ^{14}C-GFF – ^{14}C-PC). Results from this comparison provided information on whether differences in retention of ^{14}C-organic carbon by glass fiber and polycarbonate filters in our time-series measurements might reflect differences in the volume of seawater filtered for these measurements (i.e., 500 ml onto glass fiber vs. 100 ml onto polycarbonate filters).

Contextual Biogeochemical Analyses

Seawater samples for measurements of nutrient concentrations (nitrate + nitrite, N + N; soluble reactive phosphorus, SRP) were collected in 125 or 500 ml acid-washed polyethylene bottles and stored upright in a freezer for analysis on shore. Concentrations of N + N were determined using the high sensitivity chemiluminescent technique (Garside, 1982; Dore and Karl, 1996); SRP concentrations were analyzed using the magnesium-induced co-precipitation (MAGIC) method (Karl and Tien, 1992). Daily fluxes of photosynthetically active radiation (PAR; 400 to 700 nm wavelength) were measured on HOT cruises using a deckboard LI-COR LI-192 cosine collector. Vertical profiles of downwelling PAR were measured daily at noon using a Satlantic HyperPro radiometer. Coincident measurements of incident PAR were collected using a deckboard radiometer (Satlantic); these measurements were used to derive diffuse attenuation coefficients (K_{PAR}) for each cruise. Derived K_{PAR} values, together with daily integrated incident PAR measurements, were utilized to compute daily downwelling flux of PAR at discrete depths.

Data Analyses and Statistics

We evaluated seasonality in upper ocean properties and rates of ^{14}C-productivity by binning our data into predefined seasons based on the solstices and equinoxes (i.e., Spring: March 20 to June 20; Summer: June 21 to September 22; Fall: September 23 to December 20; and Winter: December 21 to March 19). Analysis of variance (ANOVA) was used to examine possible seasonality in vertically-binned (0–45 m and 75–125 m) volumetric rates of ^{14}C-GFF, ^{14}C-PC, and ^{14}C-DOC. We also examined temporal variability in rates of productivity using various time-series statistical models, including an optimized least squares monthly regression approach described in Llope et al. (2007), and the Lomb-Scargle periodogram for unevenly sampled time-series data (Scargle, 1982). We first used these techniques to test for seasonality in the near-monthly, depth-integrated (0–75 m) HOT ^{14}C-GFF measurements collected between 1989 and 2013 (see http://hahana.soest.hawaii.edu/hot/hot-dogs/interface.html). We then examined the time-series of rate measurements (^{14}C-GFF, ^{14}C-PC, and ^{14}C-DOC) from the two periods (i.e., October 2004 to October 2007 and April 2010 to October 2012) sampled in the current study using these statistical techniques. The Lomb-Scargle periodogram analysis was performed in the R statistical environment (R Development Core Team, 2008) using the "lomb" package (Ruf, 1999). The Llope et al. (2007) model was fit to the data using MATLAB (MathWorks). Depth-integrated rates and stocks were calculated using trapezoidal integration. Data were tested for normality, and if not normally distributed, were $\log_{10}$ transformed; when transformed data failed to conform to the assumption of normality, nonparametric statistical methods were utilized. For statistical analyses of ratios (e.g., ^{14}C-DOC: ^{14}C-PC), geometric rather than arithmetic

means and standard deviations were used (Zar, 1999). For computing mean rates of ^{14}C-DOC, measured rates falling below the limit of detection were designated as having a value of zero.

To evaluate the relationship between *in situ* PAR and measured rates of productivity, the derived daily PAR fluxes and measured rates of production were fitted to photosynthetic-irradiance (P-E) relationships using the equation of Platt et al. (1980):

$$P = P_{max}\left[1 - \exp\left(-\alpha E/P_{max}\right)\right] \exp\left(-\beta E/ P_{max}\right) \quad (1)$$

where P is the rate of carbon fixation, P_{max} is the maximum rate of photosynthesis without photoinhibition, E is the light flux (PAR), α is the initial slope of the curve (representing the rate of maximum light utilization), and β is the rate of photoinhibition. These relationships were examined for rates of ^{14}C-GFF, ^{14}C-PC, and ^{14}C-DOC. From these relationships values for E_k (the irradiance necessary to saturate carbon fixation) were calculated as follows:

$$E_k = P_{max}/\alpha \quad (2)$$

HOT program measurements utilized in this study (nutrients, PAR, chlorophyll *a*, and rates of ^{14}C-GFF production) are available via the HOT program data website (http://hahana.soest.hawaii.edu/hot/hot-dogs/). Rates of ^{14}C-DOC and ^{14}C-PC are available via the Center for Microbial Oceanography: Research and Education (C-MORE) data system (http://cmore.soest.hawaii.edu/datasearch/data.php).

Results

Biogeochemical Context

Consistent with HOT program sampling of Station ALOHA, upper ocean concentrations of inorganic nutrients were very low throughout the period of this study, with near-surface (5 m) concentrations of N + N persistently < 3 nM and SRP averaging 66 nM. In the dimly-lit regions of the lower euphotic zone (100–125 m) concentrations of N + N increased and became more variable, ranging between 0.2 and 3.0 μM (**Figure 1**). The penetration of PAR decreased more than two orders of magnitude through the upper 125 m of the water, with fluxes at the sea surface ranging from ∼11 to 57 mol quanta m^{-2} d^{-1} and decreasing to 0.02–0.8 mol quanta m^{-2} d^{-1} by 125 m. Incident PAR also demonstrated significant seasonal variability (One-Way ANOVA, $p < 0.0001$), with fluxes ranging between 11 and 42 mol quanta m^{-2} d^{-1} in the winter, increasing approximately 2-fold (on average) in the summer (ranging from 32 to 57 mol quanta m^{-2} d^{-1}; **Table 1**). Concentrations of chlorophyll *a* were consistently elevated in the lower euphotic zone (70–140 m; **Figure 1**).

Measurements of ^{14}C-productivity and Chlorophyll *a*

We examined vertical variability associated with ^{14}C- based productivity at Station ALOHA over the two time periods sampled as part of the current study (October 2004–October 2007 and April 2010–October 2012). Rates of ^{14}C-PC and HOT program measurements of ^{14}C-GFF demonstrated similar depth-dependent patterns and temporal variability. Average rates

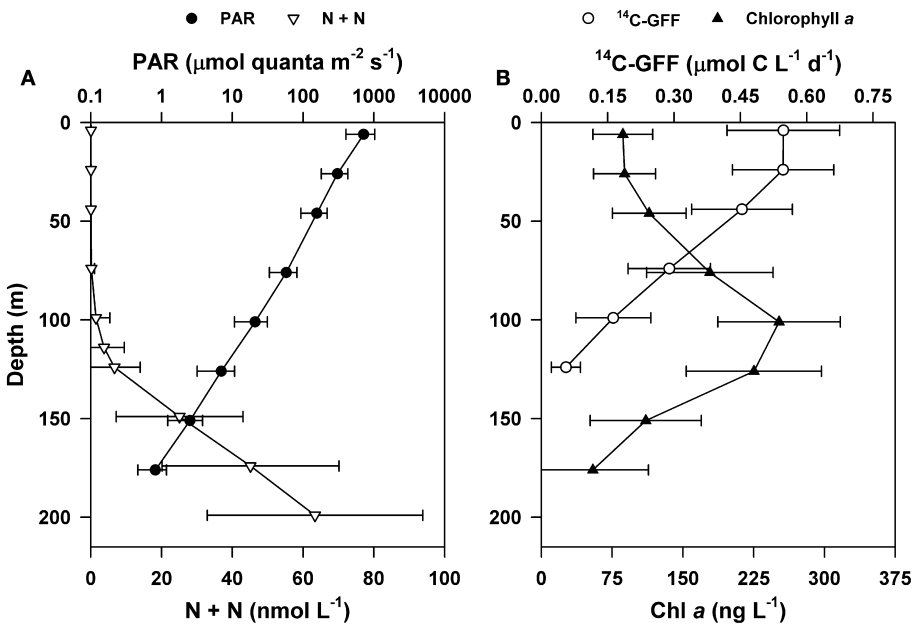

FIGURE 1 | Depth profiles of mean upper ocean properties at Station ALOHA during the period of study (2004–2013). (A) Concentrations of nitrate + nitrite (N + N; open triangles) and flux of photosynthetically active radiation (PAR; closed circles). **(B)** Concentrations of chlorophyll *a* (Chl *a*; closed triangles) and rates of ^{14}C-GFF based primary production (open circles). Error bars are ± 1 standard deviation of the time-averaged means.

TABLE 1 | Seasonally averaged (± standard deviations) rates of productivity and irradiance for the two time periods of this study.

Season	October 2004–October 2007			April 2010–October 2012				
	PAR (mol quanta m^{-2} d^{-1})	^{14}C-GFF (μmol C L^{-1} d^{-1})	^{14}C-PC (μmol C L^{-1} d^{-1})	PAR (mol quanta m^{-2} d^{-1})	^{14}C-GFF (μmol C L^{-1} d^{-1})	^{14}C-PC (μmol C L^{-1} d^{-1})	^{14}C-DOC (μmol C L^{-1} d^{-1})	%PER
Winter	21.0 ± 7.5	0.45 ± 0.09	0.28 ± 0.05	29.9 ± 3.6	0.58 ± 0.12	0.37 ± 0.11	0.11 ± 0.11	23.78 ± 14.28
0–45 m	(n = 7) B••	(n = 18) B•	(n = 18) B••	(n = 4) B*	(n = 15) A	(n = 15) A	(n = 11) A	(n = 11) A
Spring	47.8 ± 4.0	0.52 ± 0.07	0.29 ± 0.06	39.4 ± 10.1	0.51 ± 0.10	0.30 ± 0.07	0.06 ± 0.06	11.94 ± 12.79
0–45 m	(n = 7) A••	(n = 21) AB	(n = 21) B••	(n = 6) AB	(n = 18) A	(n = 18) A	(n = 21) A	(n = 19) A
Summer	46.3 ± 6.9	0.56 ± 0.14	0.38 ± 0.10	43.4 ± 2.8	0.42 ± 0.08	0.28 ± 0.04	0.07 ± 0.04	17.66 ± 10.3
0–45 m	(n = 8) A••	(n = 24) A•	(n = 24) A••	(n = 8) A*	(n = 27) A	(n = 27) A	(n = 25) A	(n = 25) A
Fall	30.8 ± 5.7	0.46 ± 0.10	0.27 ± 0.05	29.3 ± 9.9	0.49 ± 0.09	0.28 ± 0.06	0.05 ± 0.04	11.57 ± 9.77
0–45 m	(n = 9) B••	(n = 27) B•	(n = 27) B••	(n = 5) B*	(n = 18) A	(n = 21) A	(n = 15) A	(n = 15) A
Winter	0.69 ± 0.25	0.13 ± 0.12	0.08 ± 0.08	1.04 ± 0.30	0.22 ± 0.13	0.13 ± 0.09	0.03 ± 0.04	18.98 ± 20.32
75–125 m	(n = 7) C•	(n = 18) B**	(n = 18) B*	(n = 3) A	(n = 15) A	(n = 15) A	(n = 12) A	(n = 11) A
Spring	1.83 ± 0.50	0.23 ± 0.12	0.14 ± 0.08	1.85 ± 0.62	0.16 ± 0.13	0.10 ± 0.08	0.02 ± 0.05	12.63 ± 20.54
75–125 m	(n = 7) A•	(n = 21) A**	(n = 21) A*	(n = 6) A	(n = 18) A	(n = 18) A	(n = 18) A	(n = 18) A
Summer	1.42 ± 0.66	0.18 ± 0.13	0.11 ± 0.08	1.95 ± 0.88	0.11 ± 0.09	0.08 ± 0.06	0.04 ± 0.04	18.43 ± 21.23
75–125 m	(n = 6) AB	(n = 24) AB	(n = 24) AB	(n = 8) A	(n = 27) A	(n = 27) A	(n = 24) A	(n = 23) A
Fall	1.11 ± 0.28	0.13 ± 0.11	0.08 ± 0.07	2.09 ± 1.44	0.20 ± 0.15	0.12 ± 0.10	0.02 ± 0.05	12.32 ± 23.65
75–125 m	(n = 9) BC	(n = 27) B**	(n = 27) B*	(n = 5) A	(n = 18) A	(n = 18) A	(n = 15) A	(n = 12) A

*Mean irradiance (PAR) at the sea surface and at the 75 m depth horizon and vertically-binned (0–45 and 75–125 m) rates of ^{14}C-GFF and ^{14}C-PC from October 2004–October 2007, and from April 2010–October 2012. Also shown are vertically-binned ^{14}C-DOC and %PER (^{14}C-DOC / (^{14}C-DOC + ^{14}C-PC)) from April 2010–October 2012. Number of measurements (n) used to compute seasonal means shown in parentheses. Mean values denoted with the same letter are statistically indistinguishable. P-values are denoted with *$p < 0.05$, **$p < 0.005$, •$p<0.001$, and ••$p < 0.0001$.*

of ^{14}C-PC and ^{14}C-GFF decreased ∼3-fold between the well-lit upper ocean waters (<45 m; average PAR flux at 45 m of 4.1 ± 1.7 mol quanta m^{-2} d^{-1}) and the lower euphotic zone (75–125 m; **Figure 2**). HOT program measurements of ^{14}C-GFF averaged 0.52 ± 0.12 μmol C L^{-1} d^{-1} in the upper euphotic zone, decreasing and becoming more temporally variable (0.17 ± 0.13 μmol C L^{-1} d^{-1}) in the light-limited regions of the lower euphotic zone (**Figures 2**, **3**). Upper euphotic zone ^{14}C-PC rates averaged 0.32 ± 0.08 μmol C L^{-1} d^{-1} and decreased to a mean value of 0.10 ± 0.07 μmol C L^{-1} d^{-1} near the base of the euphotic zone (**Figures 2**, **3**). Both volumetric and depth-integrated (0–125 m) rates of ^{14}C-GFF were significantly greater (by ∼1.7-fold, on average) than coincident measurements of ^{14}C-PC (**Figure 2**; Kruskal-Wallis, $p < 0.0001$).

By comparing concentrations of chlorophyll *a* and rates of ^{14}C-productivity measured using polycarbonate filters to coincident HOT program measurements made using glass fiber filters we were able to examine differences in the retention characteristics of these two types of filters (**Figure 4**). This comparison revealed that volumetric concentrations and vertically integrated (0–125 m) inventories of chlorophyll *a* derived from both polycarbonate and glass fiber filters were statistically indistinguishable (Kruskal-Wallis, $p > 0.05$; **Figure 4**).

To examine temporal variability in rates of ^{14}C-DOC, we utilized the methodology described by Karl et al. (1998). The resulting precision of the derived rates, based on the coefficient of variation of triplicate ^{14}C-DOC samples, ranged from 2 to 74% (averaging 29%). In comparison, the precision associated with the ^{14}C-PC and ^{14}C-GFF measurements ranged 0.3–70% (averaging 19%) and 0.5–50% (averaging 10%), respectively. More than half of ^{14}C-DOC samples were above the calculated detection limit (defined as three times the standard deviation values of the mean time zero "blanks" for each cruise) in the three uppermost depths (5, 25, 45 m), but by 125 m less than 20% of ^{14}C-DOC measurements were above the detection limit (**Figure 3**, **Table 2**). In contrast, measurements of ^{14}C-PC and ^{14}C-GFF were consistently above detection limits, irrespective of the depth sampled.

Rates of ^{14}C-DOC production were consistently lower than either ^{14}C-PC or ^{14}C-GFF (**Figure 2**). Upper ocean rates of ^{14}C-DOC production averaged 0.07 ± 0.05 μmol C L^{-1} d^{-1} and decreased to 0.03 ± 0.04 μmol C L^{-1} d^{-1} in the lower euphotic zone; however, rates of ^{14}C-DOC in the lower euphotic were frequently below detection (**Table 2**). The resulting depth-dependent decreases in rates of ^{14}C-DOC were slightly less (∼2.4-fold) than observed for either ^{14}C-PC or ^{14}C-GFF. Rates of ^{14}C-DOC in the upper euphotic zone averaged ∼21% of ^{14}C-PC, while mean rates of ^{14}C-DOC in the lower euphotic zone were equivalent to ∼33% of ^{14}C-PC (**Figure 2**). The resulting sum of the ^{14}C-PC and ^{14}C-DOC was consistently lower than the HOT program measurements of ^{14}C-GFF; on average, the ^{14}C-GFF rates were 1.4-fold greater than the sum of the coincident ^{14}C-PC and ^{14}C-DOC measurements.

We evaluated potential relationships between depth-dependent changes in PAR and the various ^{14}C-based

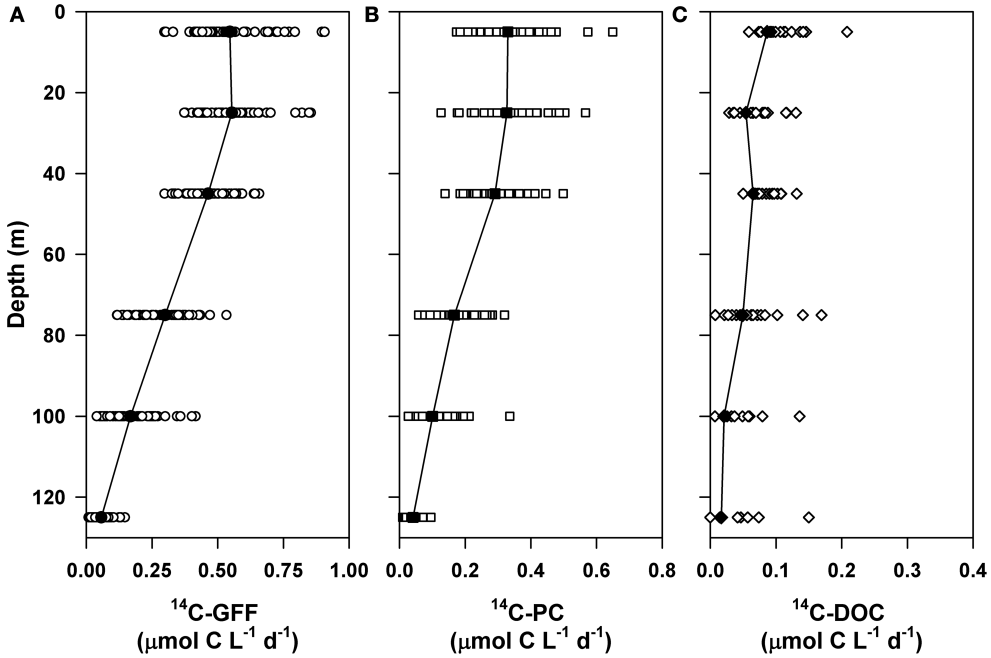

FIGURE 2 | Vertical profiles of rates of ^{14}C-based primary production filtered onto (A) glass fiber filters (^{14}C-GFF) and (B) 0.2 μm polycarbonate membrane filters (^{14}C-PC). Also shown are **(C)** rates of ^{14}C-DOC production.

measurements of productivity using a hyperbolic photosynthesis-irradiance model (Platt et al., 1980). Although the model provided information on vertical relationships between ^{14}C-GFF, ^{14}C-PC and the downwelling light field, the relationship between light intensity and rates of ^{14}C-DOC productivity was poorly described using this model (**Table 3**, **Figure 5**). Rates of both ^{14}C-PC and ^{14}C-GFF demonstrated similar patterns as a function of irradiance, increasing linearly with increasing light intensity in the lower euphotic zone, and saturating at light intensities (E_K) ~1.5 mol quanta m^{-2} d^{-1} (**Table 3**). Throughout the study, the 1.5 mol quanta m^{-2} d^{-1} isolume varied between 35 and 97 m. Neither ^{14}C-GFF nor ^{14}C-PC demonstrated significant photoinhibition (**Table 3**). The initial slope (α) derived from the relationship between ^{14}C-GFF and PAR was significantly greater than that derived from the relationship of ^{14}C-PC to PAR (**Table 3**).

Temporal Variability in Rates of ^{14}C-based Productivity

We examined temporal variability in the resulting time-series measurements of ^{14}C-GFF, ^{14}C-PC, and ^{14}C-DOC productivity. As a result of the low detectability of ^{14}C-DOC production rates in the lower euphotic zone, we confined our analysis of time variability in productivity to the upper 75 m. Depth-integrated (0–75 m) rates of ^{14}C-GFF ranged between 21.8 and 48.7 mmol C m^{-2} d^{-1} (**Figure 6**) throughout the study, while rates (0–75 m) of ^{14}C-PC production ranged between 11.4 and 31.5 mmol C m^{-2} d^{-1} (**Figure 6**). Rates of ^{14}C-DOC productivity ranged from undetectable to 12.6 mmol C m^{-2} d^{-1} (**Figure 6**), and did not vary significantly with time-varying changes in ^{14}C-GFF (Model II linear regression; $r^2 = 0.01$, $p > 0.2$), rates of ^{14}C-PC (Model II linear regression; $r = 0.00$, $p > 0.4$), or with the resulting differences in derived rates of ^{14}C-production (^{14}C-delta $= ^{14}$C-GFF $- ^{14}$C-PC) (Model II linear regression; $r = 0.06$, $p > 0.15$). The resulting depth-integrated rates of ^{14}C-DOC were temporally variable, with rates varying ~9-fold (1.4 to 12.6 mmol C m^{-2} d^{-1}) over the period of study, while rates of ^{14}C-delta varied ~11-fold (2.6 to 27.8 mmol C m^{-2} d^{-1}). In contrast, rates of ^{14}C-GFF and ^{14}C-PC varied by ~2 and ~3-fold, respectively (**Figure 6**).

Binning our measurements by predefined seasons and examining possible seasonality in volumetric rates of ^{14}C-DOC, ^{14}C-PC, and ^{14}C-GFF in both the well-lit, upper ocean (0–45 m) and dimly-lit, lower euphotic zone (75–125 m) highlighted apparent seasonal differences among the measures of productivity. When combining all the data collected for this study (October 2004–October 2007 and April 2010–October 2012), rates of both ^{14}C-PC and ^{14}C-GFF in the upper euphotic zone were significantly greater during the summer than during the winter (One-Way ANOVA; $p < 0.01$ and $p < 0.05$, respectively), while rates of ^{14}C-DOC demonstrated no significant seasonality (One-Way ANOVA; $p > 0.05$). In the lower euphotic zone, rates of ^{14}C-GFF were greater in the spring than during fall and winter (One-Way ANOVA; $p < 0.0005$), while rates in the summer were greater than rates measured in the fall (One-Way ANOVA; $p < 0.0005$). Lower euphotic zone ^{14}C-PC rates were greater during the spring than during fall and winter, while rates of ^{14}C-DOC were not significantly different among seasons (One-Way ANOVA; $p < 0.005$). However, when we considered the two periods measured during this study (**Table 1**), seasonal

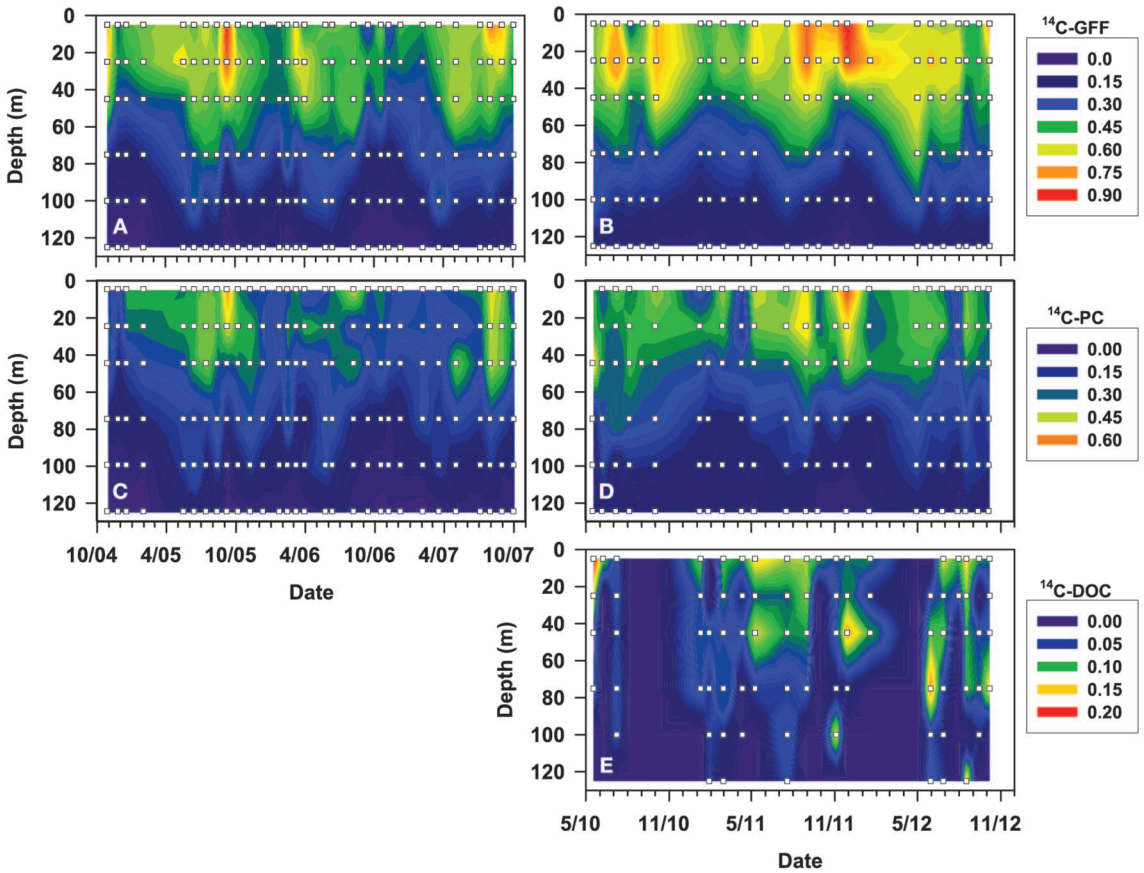

FIGURE 3 | Contour plots depicting vertical distributions of rates of ^{14}C-GFF (A,B), ^{14}C-PC (C,D), and ^{14}C-DOC (E), in μmol C L^{-1} d^{-1}, over the time periods (October 2004–October 2007 and April 2010–October 2012) evaluated for this study. White squares depict depths and dates where detectable rates were measured.

differences were only observed during the first period of this study. These differences were similar to those observed when both periods were considered together. In contrast, no significant differences were seen for any rates measured during the second period of observations (One-Way ANOVA; $p > 0.05$).

Results of the seasonal comparisons led us to analyze the resulting time-series measurements of ^{14}C-DOC, ^{14}C-delta, ^{14}C-PC, and ^{14}C-GFF using two different time-series statistical models. Application of the Lomb-Scargle periodogram (Ruf, 1999) to the full ^{14}C-GFF time-series (1989–2013) revealed a significant periodicity at ∼12 months ($p < 0.0000005$; data not shown), consistent with previously described annual cycle of primary productivity at Station ALOHA, where rates increase in summer compared to winter (Karl et al., 1996; Letelier et al., 1996; Church et al., 2013). However, use of the Lomb-Scargle periodogram to analyze the time-series rates (^{14}C-DOC, ^{14}C-PC, and ^{14}C-GFF) either as a combined record (i.e., October 2004–October 2007 and April 2010–October 2012) or either record alone identified no significant periodicity ($p > 0.05$). Use of the model described in Llope et al. (2007) revealed similar results. Results from both the seasonal regression and the Lomb-Scargle periodogram suggested that the measurement records made during this study were of insufficient length to identify recurring temporal patterns.

Diel Variability in Rates of Productivity

We conducted three experiments designed to evaluate short-term (daily scale) variability in the various measures of ^{14}C-productivity. For these experiments, we varied the incubation period for the ^{14}C measurements, including samples incubated during the morning hours only (predawn to noon), the full photoperiod (predawn to dusk), and over a full 24 h period (predawn to the following predawn). These experiments demonstrated significant overnight losses of particulate ^{14}C-labeled carbon relative to incubations conducted throughout the photoperiod (**Figure 7**). Hourly rates measured during morning hours only were not significantly different from hourly rates measured during the entire photoperiod for ^{14}C-PC (One-Way ANOVA; $p > 0.5$). In contrast, hourly rates measured during the photoperiod were greater than those measured over the full 24 h for both ^{14}C-GFF and ^{14}C-PC (One-Way ANOVA; $p < 0.001$). The resulting hourly rates of ^{14}C-PC and ^{14}C-GFF production measured over a 24 h period were 37 ± 16% and 43 ± 17% of rates measured over the photoperiod, hence

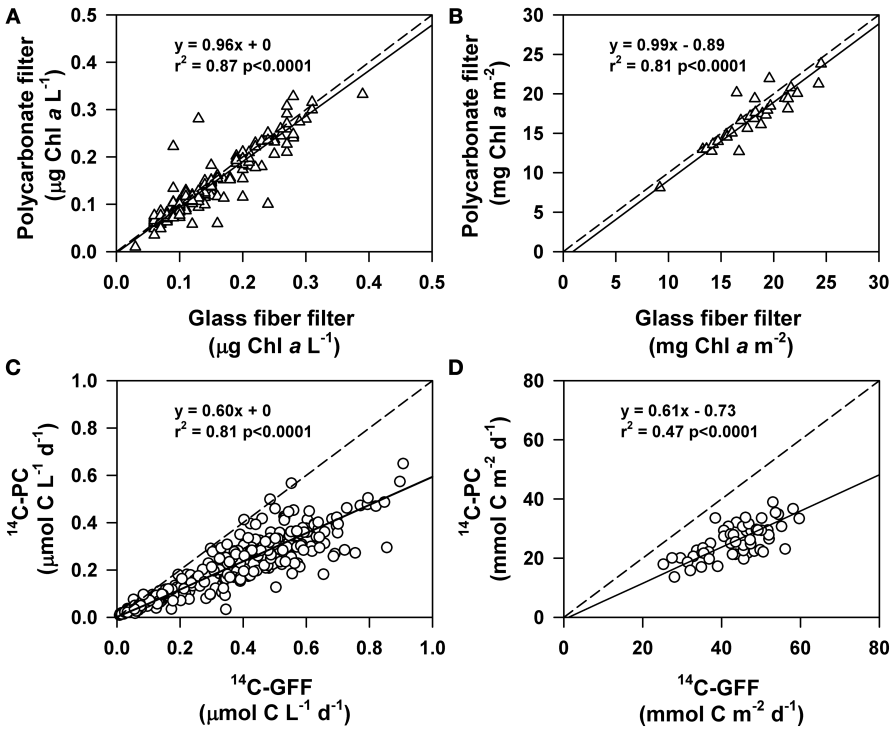

FIGURE 4 | Comparison of measurements of chlorophyll *a* and ^{14}C-primary production on either glass fiber or polycarbonate filters. Solid lines are Model II (geometric mean) linear regressions, while dashed lines depict the 1:1 ratio. Shown on each plot are the regression equation, the r^2 of the relationship, and the *p*-value for **(A)** volumetric measurements of chlorophyll *a*, **(B)** depth-integrated (0–125 m) chlorophyll *a*, **(C)** volumetric measurements of ^{14}C-primary production, and **(D)** depth-integrated (0–125 m) measurements of ^{14}C-primary production.

TABLE 2 | Mean (± standard deviation; stdev) of ^{14}C-DOC concentrations measured at the beginning (time zero; T_0) and end of incubation period (time final; T_f) at six euphotic zone depths evaluated in this study.

Depth (m)	Mean ± stdev ^{14}C-DOC T_0(μmol C L^{-1})	Mean ± stdev ^{14}C-DOC T_f(μmol C L^{-1})	Mean ± stdev T_f:T_0	% Detectable
5	0.07 ± 0.06 (*n* = 25)	0.15 ± 0.06 (*n* = 69)	2.87 ± 1.57	71%
25	0.08 ± 0.07 (*n* = 24)	0.14 ± 0.06 (*n* = 69)	2.54 ± 1.68	70%
45	0.06 ± 0.04 (*n* = 25)	0.12 ± 0.06 (*n* = 69)	2.30 ± 1.48	60%
75	0.09 ± 0.12 (*n* = 23)	0.10 ± 0.07 (*n* = 68)	1.73 ± 1.55	38%
100	0.08 ± 0.10 (*n* = 23)	0.08 ± 0.05 (*n* = 70)	1.26 ± 0.67	29%
125	0.09 ± 0.08 (*n* = 23)	0.07 ± 0.06 (*n* = 64)	1.06 ± 0.56	17%

The percent detectable indicates the proportion of the T_f samples that were greater than three times the standard deviation of the mean T_0 (defined as the limit of detection). Incubation times for T_f measurements ranged from 11 to 13 h (full photoperiod). Shown in parentheses are number of measurements (n) used to compute means.

the amount of carbon fixed over 24 h averaged 75 ± 15% and 87 ± 8% of photoperiod carbon fixation for ^{14}C-PC and ^{14}C-GFF, respectively. In contrast, hourly rates of ^{14}C-DOC and ^{14}C-GFF were significantly greater for incubations during the morning hours (One-Way ANOVA; $p < 0.05$) than for incubations lasting the full photoperiod; photoperiod rates of ^{14}C-DOC were 43 ± 17% of hourly rates measured during morning hours only. Rates of ^{14}C-DOC measured over a 24 h period were not significantly different from rates measured over the photoperiod (One-Way ANOVA; $p > 0.10$).

^{14}C-DOC Retention by Filter Type

We conducted several experiments designed to evaluate possible reasons for the greater retention of ^{14}C-organic carbon on glass fiber filters relative to polycarbonate filters. The first set of experiments involved stacking polycarbonate and glass fiber filters in series for subsequent filtration of ^{14}C-labeled whole seawater samples. When a glass fiber filter was stacked on top of a polycarbonate filter, the polycarbonate filter retained a small fraction (<5%) of the total ^{14}C activity associated with both filters (**Supplementary Figure 1**). In contrast, when a polycarbonate filter was overlaid on a glass fiber filter, the glass fiber filter retained >33% of the resulting total ^{14}C-activity. Similar experiments conducted to examine filter retention of chlorophyll *a* through stacked glass fiber and polycarbonate filters revealed that irrespective of which filter type was on top, the bottom filter retained <5% of the measured chlorophyll *a* (**Supplementary Figure 1**) of the top filter. Another experiment was conducted to evaluate successive retention of ^{14}C-organic

TABLE 3 | Descriptive characteristics of production-irradiance curve fitting results based on the Platt et al. (1980) model.

Rate	Curve fitting significance	P_{max}	P_{max} S.E.	α	α S.E.	β	E_k	E_k S.E.
^{14}C-GFF	$p < 0.0001, r^2 = 0.82$	0.53[a]	0.02	0.35[ac]	0.02	0.00[b]	1.5	0.10
^{14}C-PC	$p < 0.0001, r^2 = 0.74$	0.31[a]	0.01	0.21[a]	0.02	0.00[b]	1.5	0.12
^{14}C-DOC	$p > 0.05, r^2 = 0.12$	0.09[a]	0.01	0.15[b]	0.08	0.00[b]	6.2	3.3

Parameters derived for ^{14}C-GFF and ^{14}C-PC from data collected October 2004–October 2007 and April 2010–October 2012; ^{14}C-DOC parameters from data collected April 2010–April 2013. Significance (p-value) and coefficients of determination (r^2) for regression analyses are shown, along with derived parameters and standard error (S.E.). Units for P_{max}, α, β, and E_k are μmol C L^{-1} d^{-1}, μmol C L^{-1} d^{-1} (mol quanta m^{-2} d$^{-1)-1}$, μmol C L^{-1} d^{-1} (mol quanta m^{-2} d$^{-1)-1}$, and mol quanta m^{-2} d^{-1}, respectively.

[a] $p < 0.0001$.

[b] $p > 0.05$.

[c] *α derived for ^{14}C-GFF rates was above the 95% confidence interval of α calculated for ^{14}C-PC.*

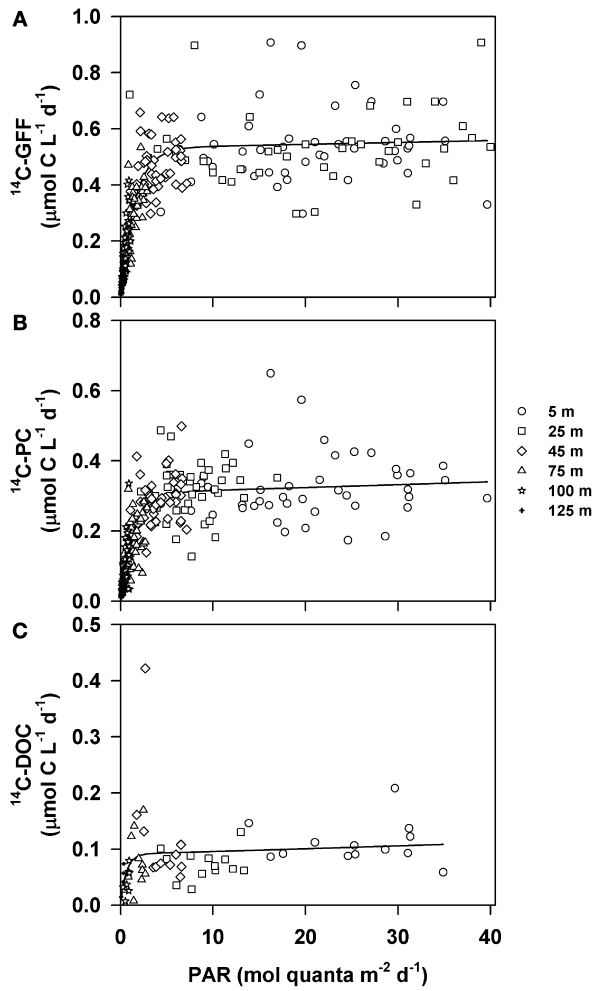

FIGURE 5 | Relationships between downwelling photosynthetically active radiation (PAR) and rates of (A) ^{14}C-GFF; (B) ^{14}C-PC; and (C) ^{14}C-DOC. Circles represent data points collected from 5 m, squares from 25 m, diamonds from 45 m, triangles from 75 m, stars 100 m and crosses from 125 m. Lines depict least-squares regression fits to the measured production rates and PAR using the Platt et al. (1980) formulation. Parameters describing the regression fits are provided in **Table 2**.

carbon by glass fiber filters. We refiltered 100 ml volumes of 0.2 μm ^{14}C-PC filtrate onto a succession of glass fiber filters and found that retention of ^{14}C labeled organic carbon by these filters decreased ∼5-fold (to <20% of the first 100 ml filtration) by the second filtration and ∼8-fold (to 13% of the first 100 ml filtration) by the fourth filtration (**Supplementary Figure 1**). Additionally, the amount of ^{14}C-DOC adsorbed onto the glass fiber filters following filtration of the first 100 ml of sample was not significantly different than the amount of ^{14}C-DOC adsorbed after filtration of 500 ml (One-Way ANOVA; $p > 0.05$; **Supplementary Figure 1**) onto one filter. We also conducted an experiment to evaluate the effects of filtering different volumes of seawater onto both glass fiber and 0.2 μm polycarbonate filters (**Supplementary Figure 2**); the resulting differences in derived rates of ^{14}C-delta did not vary with increasing volume filtered onto glass fiber filters, suggesting that the observed differences between the time-series based rates of ^{14}C-GFF and ^{14}C-PC was not an artifact of differences in filtration volumes used for these filters (500 vs. 100 ml, respectively; **Supplementary Figure 2**).

Discussion

The partitioning of organic carbon production between dissolved and particulate phases has important biogeochemical and ecological implications, and numerous hypotheses have been proposed to explain processes regulating the magnitude and variability of this partitioning (see review by Carlson, 2002). While photosynthetic production of particulate organic carbon (i.e., cellular material) can fuel numerous food web pathways (e.g., predation, viral lysis) and constitute a pathway for organic carbon export from the upper ocean (via gravitational settling or zooplankton migration), production of DOC subsidizes the energetic and nutritional demands of heterotrophic bacteria, fueling a grazing intensive microbial loop (Azam et al., 1983). Moreover, DOC can constitute an important component of carbon export, via removal through physical processes such as mixing and eddy diffusivity (Carlson et al., 1994; Emerson, 2014). Hence, quantifying the partitioning of organic carbon productivity through these distinct pathways is important for insight into the fate of recently fixed carbon through aquatic ecosystems.

In the current study, we examined rates of dissolved and particulate ^{14}C-based measures of primary production at Station ALOHA. The resulting time-series measurements yielded insight into methodological considerations underlying application of the ^{14}C-based production assays, and highlighted vertical and temporal variability in the partitioning of recently fixed carbon

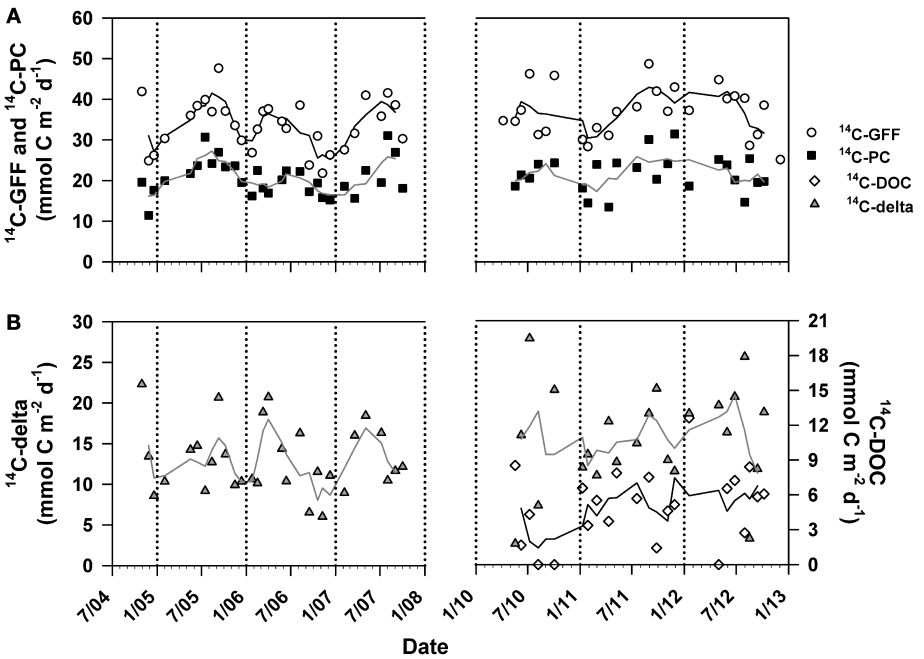

FIGURE 6 | Time-series measurements of depth-integrated (0–75 m) rates of (A) ^{14}C-GFF (open circles) and ^{14}C-PC production (closed squares). Also depicted are depth-integrated (0–75 m) rates of **(B)** ^{14}C-delta production (^{14}C-GFF—^{14}C-PC; gray triangles) and ^{14}C-DOC production (open diamonds). Lines are 3-point running means. Break in time-series indicate period where measurements were not conducted. Rates of ^{14}C-DOC below detection were considered to be equal to zero.

between particulate and dissolved pools in this ecosystem. Consistent with previous reports (Maske and Garcia-Mendoza, 1994; Karl et al., 1998; Morán et al., 1999), our study demonstrated greater (1.6-fold on average) ^{14}C-productivity derived from samples filtered onto glass fiber filters (with a nominal pore size of 0.7 μm) relative to productivity rates derived from 0.2 μm polycarbonate membrane filters. In contrast, but consistent with a previous study (Chavez et al., 1995), simultaneous fluorometric determinations of chlorophyll *a* concentrations filtered onto polycarbonate and glass fiber filters revealed no consistent difference between these filter types for retention of chlorophyll *a*, suggesting the observed differences between ^{14}C-GFF and ^{14}C-PC did not derive from differences in the efficiency with which these filters trap photosynthetic organisms.

Various studies have documented adsorption of DOC by glass fiber filters (Abdel-Moati, 1990; Maske and Garcia-Mendoza, 1994), and our findings largely support the hypothesis that the greater rates of ^{14}C-GFF relative to ^{14}C-PC derive from adsorption of ^{14}C-DOC produced during the incubation to the glass fiber filters (Karl et al., 1998; Morán et al., 1999). Notably, on average, rates of ^{14}C-GFF productivity exceeded the sum of the independent measurements of ^{14}C-PC and ^{14}C-DOC production by ∼42%. Such differences between rates of ^{14}C-PC + ^{14}C-DOC and ^{14}C-GFF may reflect incomplete recovery of ^{14}C-DOC by the oxidation and trapping procedure, loss of volatile ^{14}C-DOC during the active bubbling procedure in the ^{14}C-DOC assay, incomplete oxidation of ^{14}C-DOC by the persulfate oxidation procedure, or incomplete removal of ^{14}C-DIC from the glass fiber filters (Mague et al., 1980). Moreover, the relatively large methodological uncertainty of the ^{14}C-DOC assay (coefficient of variation of triplicate measurements averaged 29%) further complicates this comparison. Time zero blanks indicated that the amount of ^{14}C remaining on glass fiber filters post-acidification was always less than 5% of total ^{14}C retained on the these filters post-incubation. These results, together with our experiments examining retention of ^{14}C organic matter onto glass fiber filters using particle-free seawater, suggest the differences between measured ^{14}C-GFF and ^{14}C-PC reflect adsorption of ^{14}C-DOC by glass fiber filters. Hence we computed the difference between the ^{14}C-GFF and ^{14}C-PC rate measurements (^{14}C-delta) as an additional proxy for ^{14}C-DOC production. Throughout our study, there was no relationship between depth-integrated rates of ^{14}C-DOC and ^{14}C-delta, with ^{14}C-delta rates greater than ^{14}C-DOC rates on all but one cruise.

The observed differences between the ^{14}C-PC and ^{14}C-GFF rates could derive from differences in the volumes filtered for these analyses (see Huete-Ortega et al., 2012). For the ^{14}C-PC filtrations, we concentrated 100 ml of seawater sample onto polycarbonate filters, while the HOT program ^{14}C-GFF measurements rely on filtration of 500 ml volumes of seawater. This difference in volume filtered could result in greater trapping of particles or ^{14}C-DOC by the glass fiber filters, which could account for the observed discrepancy between ^{14}C-GFF and ^{14}C-DOC + ^{14}C-PC. However, results from experiments we conducted examining adsorption characteristics of glass fiber

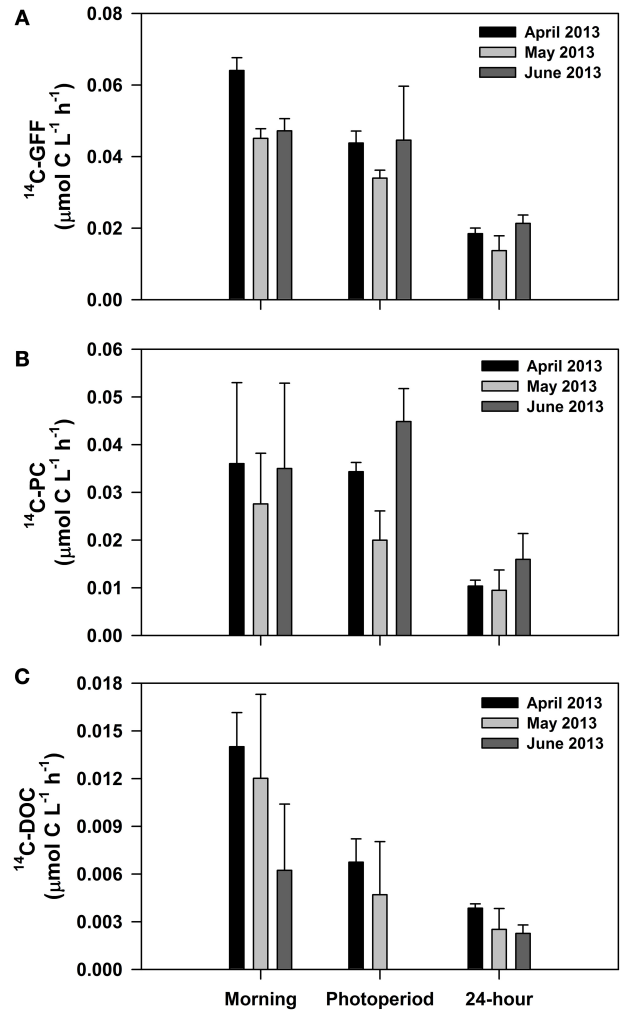

FIGURE 7 | Results from experiments conducted in April, May, and June 2013 examining daytime and nighttime changes in hourly rates of carbon fixation. Depicted are average hourly rates of **(A)** ^{14}C-GFF; **(B)** ^{14}C-PC; and **(C)** ^{14}C-DOC. All rates represent incubations initiated before dawn and terminated at different periods of the day ("morning" samples terminated at noon, "photoperiod" samples terminated at sunset, and "24-hour" samples terminated before dawn the following day).

filters suggest that the majority of ^{14}C-DOC is adsorbed during filtration of the first 100 ml of sample. Moreover, we observed no significant increases in the volume corrected differences between ^{14}C-GFF and ^{14}C-PC (^{14}C-delta) with increasing filtration volume above 100 ml. Such results suggest the differences in filtered volumes for the ^{14}C-PC and ^{14}C-GFF determinations likely would not account for the observed differences in ^{14}C-delta and direct quantification of ^{14}C-DOC. Regardless of the mechanism responsible for the apparent offset between these measurements, the coincident measurements of ^{14}C-productivity using both glass fiber and polycarbonate filters provided a useful constraint on the partitioning of primary production between dissolved and particulate phases in our study.

The resulting time-series measurements of ^{14}C-DOC, ^{14}C-GFF, and ^{14}C-PC productivity provided insight into vertical- and time-dependent variations in the partitioning of primary production among dissolved and particulate phases at Station ALOHA. Over ∼2.5 years (2010–2012), rates of ^{14}C-DOC production averaged 18% (± 10%) of the daytime photosynthetic production (sum of ^{14}C-DOC and ^{14}C-PC). Integrated rates (0–75 m) of ^{14}C-DOC production ranged approximately 8.8-fold over the period of study (1.4 to 12.6 mmol C m^{-2} d^{-1}) and did not appear to co-vary with changes in ^{14}C-GFF or ^{14}C-PC primary productivity. Similar to studies in the oligotrophic Atlantic Ocean (Teira et al., 2001, 2003), rates of ^{14}C-DOC in the current study were not well correlated (either in depth or in time) with estimates of photosynthetic particulate matter production (based on rates of ^{14}C-GFF or ^{14}C-PC). When data from both time periods of this study were combined, in both the upper and lower regions of the euphotic zone, rates of ^{14}C-GFF and ^{14}C-PC varied seasonally, with elevated production in the upper euphotic zone during the summer, while rates in the lower euphotic zone were greatest in the spring. We utilized various statistical models (the Lomb-Scargle periodogram and an optimized least squares monthly regression) to evaluate possible recurring temporal patterns in our datasets. However, the relatively limited duration of our near-monthly observations (∼2.5 years for ^{14}C-DOC, and ∼5 years for ^{14}C-GFF and ^{14}C-PC) proved insufficient to derive statistically robust patterns based on these analyses. Use of comparative statistical models (ANOVA) did highlight apparent seasonality in the longer duration time-series measurements of ^{14}C-PC and ^{14}C-GFF; however, rates of ^{14}C-DOC did not demonstrate similar seasonal fluctuations. These analyses confirmed that the latter period of our observations (2010–2012) coincided with a period of time where anomalous patterns in productivity were observed (Wilson et al., 2015). Unlike the seasonal climatology observed in the HOT program record of productivity, where rates of ^{14}C-GFF increase 2-3-fold during the summer months (Karl and Church, 2014), rates of ^{14}C-GFF were greatest during the winter months of 2011 and 2012. Such anomalous seasonal patterns in productivity likely contributed to the relatively poor fit of the various statistical models we applied to our measurements of ^{14}C-GFF, ^{14}C-PC, and ^{14}C-DOC.

The weak relationship observed between measurements of ^{14}C-DOC and rates of ^{14}C-PC and ^{14}C-GFF may reflect the complex suite of processes that control net production of DOC in this ecosystem, many of which may not be directly coupled in time to photosynthetic production of particulate carbon and likely vary with depth. Ecosystems dominated by picoplankton such as the NPSG often appear to partition a greater fraction of photosynthetic production toward DOC than do larger phytoplankton (Legendre and Rassoulzadegan, 1995; Malinsky-Rushansky and Legrand, 1996; Teira et al., 2001). The greater partitioning of fixed carbon into the DOC pool is hypothesized to derive from tightly coupled trophodynamic processes, including those linked to the functioning of the microbial loop (predation and/or viral lysis). Several studies have observed that nutrient-limited and light replete systems tend to partition a greater fraction of recently produced photosyntheate into the dissolved pool relative to the particulate pool; conversely nutrient-enriched ecosystems appear to partition a greater

fraction of the daily net productivity toward cellular (particulate) production (Carlson et al., 1998; Biddanda et al., 2001). Over the course of our study we observed significant production of ^{14}C-DOC in the persistently oligotrophic, well-lit upper euphotic zone (0–45 m); in contrast, in the light-limited but nutrient-enriched lower euphotic zone (75–125 m), rates of ^{14}C-DOC were often below our detection limit. Unlike rates of ^{14}C-DOC, rates of particulate carbon production (^{14}C-PC and ^{14}C-GFF) exhibited strong depth dependence, with rates in the dimly-lit lower euphotic zone averaging ∼33 and 34% (^{14}C-PC and ^{14}C-GFF, respectively) of rates measured in the well-lit upper ocean. As a result, the vertical distribution of PER [Percent Extracellular Release; ^{14}C-DOC / (^{14}C-DOC +^{14}C-PC)] did not demonstrate clear depth dependence, a finding consistent with previous work in more eutrophic marine systems (e.g., Marañón et al., 2004).

Application of a photosynthesis-irradiance model further emphasized the differences between vertical changes in ^{14}C-DOC compared to particulate production. Rates of ^{14}C-DOC production demonstrated no significant relationships with downwelling PAR. The observation that rates of ^{14}C-DOC varied less with depth than rates of ^{14}C-PC or ^{14}C-GFF suggests that unlike cellular production (as estimated from measurements of ^{14}C-PC and ^{14}C-GFF), light does not appear to be as strong a control on net photosynthetic production of DOC at Station ALOHA. Such results are consistent with other environmental studies conducted in ocean ecosystems (Lancelot, 1983; Marañón et al., 2004), although studies in the Gulf of Mexico and the Eastern tropical North Pacific revealed a dependence of ^{14}C-DOC production with vertical changes in irradiance (Cherrier et al., 2014). While we did not evaluate mechanisms that underlie the partitioning of photosynthetically fixed carbon into DOC, such results suggest the potential importance of processes that are light independent.

The time-series rate measurements conducted as part of this study derived from incubations lasting over the full daily photoperiod (11–13 h); during that time period, a significant fraction of the ^{14}C-DOC produced during the incubation was likely consumed. Hence our measurements of ^{14}C-DOC presumably approximate net rates of production. On three occasions, we compared ^{14}C-productivity during three time periods: morning (predawn to noon), photoperiod (predawn to dusk), and 24-h (predawn to predawn). These experiments were conducted to provide insight into nighttime removal of the recently fixed ^{14}C, and to investigate possible differences in partitioning of recently fixed ^{14}C throughout the daylight period. Rates of ^{14}C-DOC production were significantly greater during the morning compared to the full photoperiod, suggesting a larger fraction of recently fixed photosyntheate is partitioned to DOC in the morning. Alternatively, these results could reflect changes in the coupling between production and consumption throughout the day, and hence the longer duration (photoperiod) rate measurements may approximate net production while the shorter incubation may be more similar to gross ^{14}C-DOC production (e.g., Lancelot, 1979).

Our experiments demonstrated no significant difference between the total amount of ^{14}C-DOC produced during 24 h and photoperiod incubations, compared to significant nighttime losses of fixed ^{14}C-particulate carbon (^{14}C-PC and ^{14}C-GFF, respectively). Such results suggest that ^{14}C-DOC produced during the photoperiod was consumed at night at lower rates than contemporaneous particulate production; alternatively, there may be sources of ^{14}C-DOC production at night that offset simultaneous consumption. Previous results from a coastal upwelling system (Marañón et al., 2004) found no significant DOC production at night, and that study attributed DOC production to phytoplankton exudation rather than trophic processes. In contrast, in the oligotrophic NPSG, lack of significant change in ^{14}C-DOC produced at night could reflect a combination of reinforcing processes. Rapid coupling between production and consumption of ^{14}C-DOC during daylight hours could leave a relatively less reactive pool of ^{14}C-DOC to persist through the nighttime. In addition, the diverse pathways that create DOC during the day (grazing, viral lysis, direct exudation/excretion) likely continue during the night (Christaki et al., 2002; Tsai et al., 2005). In contrast, photoperiod photosynthetic production of particulate material presumably reflects net cellular production, and subsequent nighttime losses of the fixed carbon likely reflect phytoplankton respiration together with cellular removal processes (grazing, viral lysis) (Marra and Barber, 2004). Recent studies have reported diel cycle fluctuations in the physiological and transcriptional activities of both phytoplankton and heterotrophic bacteria, that appear tightly coupled (Vaulot et al., 1995; Binder and DuRand, 2002; Poretsky et al., 2009; Ottesen et al., 2013, 2014; Aylward et al., 2015). Results from the short-term productivity experiments in the present study indicate that in addition to the lack of a significant relationship between rates of particulate and dissolved primary productivity observed during our monthly scale sampling, these rates are also decoupled over daily time scales, which may have important implications for the diel activity cycles of the heterotrophic bacteria that rely on recently produced photosyntheate.

The time-series measurements of ^{14}C-primary production reported here underscore several important features of the NPSG ecosystem. Rates of net ^{14}C-DOC production appear both vertically and temporally decoupled from variations in rates of ^{14}C-GFF or ^{14}C-PC. In particular, over a ∼2.5 year period of near-monthly observations, rates of particulate matter productivity were decoupled from ^{14}C-DOC production over diel to seasonal time scales. Moreover, consistent with a prior report (Karl et al., 1998), we find that photosynthetic production of DOC can be an important, but variable pathway for organic carbon production in the NPSG, accounting for nearly one fifth of the net daytime ^{14}C-based estimates of productivity. Our results also provide indications of the complexity of interacting processes that control net production and consumption of organic matter in this ecosystem, highlighting the need for future studies quantifying the magnitude and variability of such processes.

Author Contributions

All authors contributed substantially to the design of this study, interpretation of results, and preparation of the manuscript.

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Supplementary Material

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fmars.2015.00073

Supplementary Figure 1 | Results from experiments comparing retention of ^{14}C organic matter onto either glass fiber or polycarbonate membrane filters. (A) ^{14}C-labeled primary production samples filtered either onto glass fiber filters alone, polycarbonate filters alone, glass fiber filters placed on top of polycarbonate filters (GFF/PC), or polycarbonate filters placed on top of glass fiber filters (PC/GFF); **(B)** chlorophyll *a* samples filtered across the same configuration of filters. **(C)** Sequential 100 ml re-filtrations of ^{14}C-PC filtrate onto successive new glass fiber filters (in triplicate). **(D)** Comparison of average (from triplicates) ^{14}C-DOC adsorbed to glass fiber filters from initial 100 ml filtration, sum of all five 100 ml re-filtrations (for a total volume of 500 ml, 100 ml per filter) onto five separate filters, and filtration of 500 ml onto single glass fiber filters.

Supplementary Figure 2 | Differences between ^{14}C-activity on glass fiber and polycarbonate filters (^{14}C-delta =^{14}C-GFF - ^{14}C-PC) where different volumes of seawater were filtered. ^{14}C-delta derived from paired samples where 100 ml of seawater was filtered onto polycarbonate filters, while varying volumes (100, 150, 400, and 500 ml) of seawater were filtered onto glass fiber filters. Midline of box plots indicates median value, while the upper and lower borders of the box represent the 75th and 25th percentiles, respectively.

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