Comparative study of picoplankton biomass and community structure in different provinces from subarctic to subtropical oceans

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Abstract

Picoplankton biomass and community structure in the subtropical and subarctic Pacific Oceans were investigated during November 2003, April-August 2005 and July-August 2005. The sampling covered the subarctic K2 station, the Western North Pacific subtropical Gyre (WNPG1 and 2 stations) and the Eastern North Pacific subtropical area (ENP1, 2, 3 and 4 stations). Distinct differences in community structure and autotrophic and heterotrophic picoplankton biomass were observed among the above provinces. In subtropical areas, the picoplankton community comprised *Prochlorococcus*, *Synechococcus*, picoeukaryotes and heterotrophic bacteria. While in the subarctic area (K2 station), *Prochlorococcus* were absent. *Prochlorococcus* were numerically dominant in the subtropical oceans, their abundance tended to decrease with increasing nutrient levels, which is the opposite of the other picoplankton populations. Although the aerobic anoxygenic phototrophic heterotrophic bacteria (AAPB), accounted for only a small proportion of total heterotrophic bacterial abundance, their potential contribution to carbon export may

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be important due to their larger cell size and higher cell turnover rates compared with other heterotrophic bacteria. Biomass contribution of the AAPB increased distinctly along the oligotrophic to relatively eutrophic gradient. Vertically, AAPB generally followed the phytoplankton except in the subtropical WNPG. Spatial variability of biomass in the autotrophic picoplankton was distinctly larger than that in the heterotrophic bacteria. Changes in the picoplankton community were more closely associated with latitude while nutrient availability was more important for differences in picoplankton biomass. The biomass of autotrophic picoplankton in the upper mixed-layer, and also the depth attenuation, were higher in eutrophic relative to oligotrophic waters. Picoplankton seemed to be an important source of new organic carbon for higher trohpic level organisms and for detritus production, especially in the oligotrophic subtropical gyre.

***Keywords*:** Picoplankton; Autotroph; Heterotroph; Aerobic anoxygenic phototrophic bacteria (AAPB); Subarctic ocean; Subtropical ocean

1. Introduction

Picophytoplankton (<2 μm) are composed of 3 groups of autotrophs: *Prochlorococcus*, *Synechococcus* and picoeukaryotes. These tiny primary producers contribute substantially to both total phytoplankton biomass and production in marine ecosystems, especially in oligotrophic waters where they account for up to 90% of the total photosynthetic biomass and carbon production (Campbell *et al.*, 1994; Li *et al.*, 1983). Despite a large number of ecological studies on picophytoplankton in various oceanic waters of the Pacific (Binder *et al.*, 1996; Campbell and Vaulot, 1993; Liu *et al.*, 2002a), Atlantic (Buck *et al.*, 1996; Li, 1995; Olson *et al.*, 1990), Mediterranean Sea (Bustillos-Guzman *et al.*, 1995; Vaulot *et al.*, 1990), and Arabian Sea (Campbell

et al., 1998), few studies have focused on comparisons among different marine regimes. Heterotrophic bacteria are typically considered solely as decomposers in marine ecosystems. The concept of the "microbial loop" endowed them with new roles in the biological pump (Azam *et al.*, 1983). Recent studies have further revealed that some bacteria are capable of harvesting light for supplemental energy (Yurkov and Beatty, 1998a, 1998b), such as aerobic anoxygenic phototrophic bacteria (AAPB). AAPB have been reported to play a unique role in carbon cycling in the ocean (Jiao *et al.*, 2003; Karl, 2002; Kolber *et al.*, 2001) and have drawn much attention from microbial oceanographers (Cottrell *et al.*, 2006; Schwalbach and Fuhrman, 2005; Sieracki *et al.*, 2006; Zhang and Jiao, 2007). Although the global distribution pattern of AAPB in the oceans has been brought to light (Jiao *et al.*, 2007b), differences in abundance and vertical profiles of AAPB between high latitudes and low latitudes remain unclear. In the present study, four distinct provinces in the Pacific Ocean were investigated: The western subarctic gyre (the VERTIGO station K2), the western subtropical gyre (stations WNPG1 and 2), the eastern subtropical Pacific (stations ENP1-3) and the eastern subtropical Pacific off shore waters (station ENP4). We will address differences in the picoplankton biomass and community structure between subarctic and subtropical regimes, in an attempt to better understand the mechanisms of attenuation of vertical carbon flux at different latitudes and different trophic levels.

2. Materials and methods

2.1. Study areas and sampling

Station K2, located in the Western North Pacific (47ºN 160ºE) (Fig. 1), is a relatively eutrophic site in the NW Pacific subarctic gyre, with high macronutrient levels (nutrients concentrations are provided in Buesseler *et al.* 2008), high

chlorophyll *a* concentration and significant seasonal variability in primary production and carbon export (Buesseler *et al.*, 2007; Buesseler *et al.*, 2008). Investigation at K2 was conducted during July 30 – August 6, 2005 (deployment 1, D1) and August 10 – 17, 2005 (deployment 2, D2). D1 took place during the decline of the seasonal maximum in phytoplankton biomass, and D2 was just prior to a smaller autumn bloom (Buesseler *et al.*, 2007; Buesseler *et al.*, 2008). Four vertical profiles with 5 depths within the upper 50 m water column were sampled during D1 and D2.

Stations WNPG1-2 (Fig. 1) in contrast were in oligotrophic waters in the Western North Pacific subtropical gyre, and are characterized by warm waters with persistently low macronutrients and correspondingly low surface chlorophyll (Schlitzer, 2004; Shimada *et al.*, 1993). Stations ENP1-4 (Fig. 1) were in the Eastern subtropical Pacific, which had a relatively high chlorophyll *a* concentration (Table 1) compared with WNPG (Binder *et al.*, 1996; Landry *et al.*, 1996; Schlitzer, 2004). ENP4, located in the open water off the coast, was at mesotrophic conditions among these subtropical stations (Schlitzer, 2004). Samples from stations WNPG1, WNPG2 and ENP1-3 were collected from 7-10 depths within the upper 200 m during April-August 2005. Samples from stations ENP4 were collected from 10 depths within the upper 200 m water column, and 4 deployments were conducted during November 1-15, 2003.

2.2. Hydrographic parameters

A SeaBird CTD-General Oceanic Rosette assembly with Go-Flo bottles (SBE 9/11 plus, SeaBird Inc., USA) was employed to record temperature and salinity as well as to collect seawater samples. The mixed-layer depths were defined as the maximum density gradient depth by CTD measurement. The depth of the euphotic zone was

defined as the 0.1% surface irradiance depth. Samples for chlorophyll *a* analysis were collected on 0.7 μm pore-size GF/F filter paper (Whatman) and determined using a Turner-Designs-Model 10 fluorometer. Chlorophyll *a* data at K2 were provided by the VERTIGO Project (Dr. S.I. Saitoh and S. Okamoto, Hokkaido University, Japan).

2.3 Picoplankton abundance

For picoplankton, 5 ml of seawater per tube (5 duplicate tubes for each sample) were preserved with glutaraldehyde (0.5% final concentration), quick frozen in liquid nitrogen, and then stored at -80 ºC until analysis.

Abundances of *Synechococcu*s, *Prochlorococcus*, picoeukaryotes and heterotrophic bacteria were determined using flow cytometry (FCM) (Jiao *et al.*, 2002; Marie *et al.*, 1997) with an Epics Altra II (Beckman Coulter, USA) flow cytometer, equipped with a 306C-5 argon laser (Coherent Inc., USA). 1 μm fluorescence beads (PolySciences Inc., U.S.) were added into the samples as an FCM analysis reference, and the half-peak coefficients of variation were always controlled at lower than 1.0%. The coefficients of variation in the same samples were lower than 10%. The data we used were the means.

2.4. AAPB abundance

Subsamples for AAPB analysis were collected with 100-mL brown polypropylene bottles. Immediately after sampling, aliquots of 20 mL seawater were fixed for 15 min with paraformaldehyde (2% final concentration), and then stained with 4’6-diamidino-2-phenylindole (DAPI) (5 μg mL-1, final concentration) for 30 min in the dark. Cells were filtered onto 0.2 μm pore-size black polycarbonate membranes (Whatman) for abundance determination. The subtropical samples were measured on

board. The subarctic samples were stored at -80 ºC until analysis.

An epifluorescence microscope (Carl Zeiss Axioskop) with a 50-W mercury lamp was used to image bacteria. It was equipped with an infrared-sensitive charge-coupled device camera (SPOT Diagnostic Instruments, Inc.), interfaced with a computer. Image-Pro Plus software (Media Cybernetics, Inc.) was used to detect and analyze cells in the images. AAPB abundances were determined by the time series observation based infra-red epifluorescence microscopy (TIREM) protocol (Jiao *et al.*, 2006). Cell biovolumes of AAPB and other heterotrophic bacteria were compared by image analysis using the DAPI images. For each sample, 30 AAPB cells and 30 heterotrophic bacterial cells were measured for size comparison.

2.5. Estimation of carbon biomass

Carbon biomass of the four picoplankton groups was estimated by conversion from cell abundance using the factors of 250, 53, 2100 and 20 fg C $cell^{-1}$ for *Synechococcus*, *Prochlorococcus*, picoeukaryotes and heterotrophic bacteria, respectively (Buck *et al.*, 1996; Campbell *et al.*, 1994; Lee and Fuhrman, 1987; Morel *et al.*, 1993; Simek *et al.*, 1999). The average volume of AAPB cells was 3.6±0.8 times larger than that of heterotrophic bacteria. The conversion factor for AAPB was thus determined to be 72 fg C $cell^{-1}$.

3. Results

3.1. Contrasting hydrographic conditions

K2 was characterized by low temperature, low salinity and high chlorophyll *a* concentration (Table 1). The chlorophyll maximum layer (DCM) occurred at 50 m, deeper than the mixed-layer (25m).

The subtropical stations in contrast were characterized by high temperature, high salinity and low chlorophyll *a* concentration (Table1). The surface and depth-weighted chlorophyll *a* concentrations were around 0.03 and 0.08 mg m^{-3} at the two stations in the Western North Pacific Gyre, and 0.1-0.13 mg m^{-3} at the Eastern North Pacific stations. Among stations ENP1-4, chlorophyll *a* concentration was a little higher at ENP4. DCM coincided roughly with the depth of the mixed-layer (Table 1).

3.2. Contrasting picoplankton community structure

In the subarctic area (K2 station), the picoplankton community comprised *Synechococcus*, picoeukaryotes and heterotrophic bacteria, and *Prochlorococcus* were absent. Cell abundances ranged from 1.8×10^3 to 1.1×10^4 cells ml^{-1} for picoeukaryotes and from 1.7×10^3 to 5.8×10^4 cells ml^{-1} for *Synechococcus.* Abundance of heterotrophic bacteria was about 2 orders of magnitude higher than that of picoeukaryotes. Abundance of AAPB was at the same level as *Synechococcus* (Fig. 2). Results from the two deployments (D1 & D2) showed differences in the maximum abundance depth of *Synechococcus* and picoeukaryotes over time (Fig. 2).

In the subtropical areas, the picoplankton community comprised *Prochlorococcus*, *Synechococcus*, picoeukaryotes and heterotrophic bacteria. In contrast to K2, *Prochlorococcus* were extremely abundant with depth-weighted abundances of around 8×10^4 cells ml^{-1} at WNPG station 1 and 2, 4-5 $\times10^4$ cells ml^{-1} at ENP1-3 , and 3×10^4 cells ml^{-1} at ENP4 (Table 2, Fig. 2). Among the subtropical stations, the Western North Pacific Gyre was characterized by the distinct low abundances of *Synechococcus* (10^3 cells ml^{-1}), picoeukaryotes (10^2 cells ml^{-1}) and AAPB (10^2-10^3 cells ml^{-1}). Along the ENP stations, abundance of heterotrophic bacteria increased eastward with the highest abundance of $3.2\pm0.5\times10^5$ cells ml^{-1} at ENP4 (Table 2; Fig.

2). Vertically, the maximum distribution depths of *Prochlorococcus* were always deeper than those of *Synechococcus*, picoeukaryotes and AAPB. The maximum abundance depth of *Prochlorococcus* increased with trophic conditions (Fig. 2). Such trends were less regular for other groups of picoautotrophs. The abundance of heterotrophic bacteria also decreased with depth but remained high (10^5 cells ml^{-1} at 150m) even at the bottom of the euphotic zone. The vertical distributions of AAPB were basically similar to those of the phototrophic components rather than the heterotrophic bacteria, confined to the euphotic zone (Fig. 2). The maximum abundance depths of AAPB were consistent with those for chlorophyll *a*, except for stations WNPG1-2 (Table 1). At the WNPG stations, weak maxima of AAPB were present at shallower depths than those for chlorophyll *a* (Table 1; Fig. 2).

3.3. Contrasting picoplankton carbon biomass

Off-shore station ENP4 was characterized by a remarkably high biomass of *Synechococcus* and picoeukaryotes and a low biomass of *Prochlorococcus*. In contract, stations WNPG1-2 in the subtropical gyre were characterized by an extremely high biomass of *Prochlorococcus* but a very low biomass of *Synechococcus* and picoeukaryotes (Table 2; Fig. 3). Biomass of *Synechococcus* and picoeukaryotes at K2 in the subarctic area were also significantly higher than at the other subtropical stations (except for ENP4) (Table 2; Fig. 3). From the western subtropical Pacific to the eastern subtropical Pacific, the biomass of *Prochlorococcus* decreased observably, with the highest biomass of 5.34 mgC m^{-3} at WNPG2. There was an increasing trend in biomass of both *Synechococcus* and picoeukaryotes along trophic gradients from the western to the eastern subtropical Pacific (Table 2). Although *Synechococcus* were numerically more abundant than picoeukaryotes, the latter contributed more

198 significantly to photosynthetic carbon biomass (Table 2; Fig. 3). Except for ENP4, the
199 biomass of picoeukaryotes was 2.1–2.9 times higher than that of *Synechococcus*.
200 AAPB biomass was relatively higher at K2 and lowest in the oligotrophic ocean. The
201 biomass of heterotrophic bacteria was less variable than that of pico-sized autotrophs
202 among all of the stations investigated (Table 2; Fig. 3). Higher bacterial biomass
203 usually occurred where *Synechococcus* and picoeukaryotes were more abundant. Our
204 observations were that autotrophic biomass and heterotrophic biomass of
205 picoplankton were comparable in the subtropical Western North Pacific Gyre and
206 Eastern North Pacific, while autotrophic biomass was higher than heterotrophic
207 biomass in the relatively eutrophic subarctic and the subtropical Eastern North Pacific
208 off-shore waters (Table 2).

209 There were interesting differences in the vertical distributions of carbon biomass of
210 autotrophic picoplankton and heterotrophic bacteria (Fig. 3). Carbon biomass of
211 autotrophic picoplankton in the upper mixed-layer was much higher than near the
212 bottom of the euphotic zone in relatively high nutrient and chlorophyll areas (stations
213 ENP4 and K2). While in oligotrophic waters, variations in carbon biomass of the
214 picoplankton between the upper and lower layer were much smaller (Fig. 3).

215

216 **4. Discussion**

217 Picoplankton in the Pacific Ocean has been studied over the past few decades
218 (Binder *et al.*, 1996; Campbell and Vaulot, 1993; Ishizaka *et al.*, 1994; Jiao *et al.*,
219 2005; Jiao *et al.*, 2002; Jochem, 1995; Landry *et al.*, 1996; Liu *et al.*, 2002a; Liu *et al.*,
220 2002b; Partensky *et al.*, 1996; Shimada *et al.*, 1993). The distinct differences between
221 this study and previous ones are that we compared vertical profiles of carbon biomass
222 between autotrophic and heterotrophic picoplankton across a larger-scale

environmental gradient and that we included AAPB as a unique picoplankton component and showed the variation of picoplankton community structure in different marine provinces.

The abundance of picoplankton we observed in the subarctic sea area compared favorably to the range seen by Liu *et al.* (Liu *et al.*, 2002a; Liu *et al.*, 2002b). The abundances observed in the Western North Pacific subtropical Gyre, ranging from 4.5×10^4 to 1.2×10^5 for *Prochlorococcus*, from 8.4×10^2 to 2.4×10^3 for *Synechococcus*, from 4.1×10^2 to 1.2×10^3 for picoeukaryotes and from 1.5×10^5 to 3.4×10^5 for total heterotrophic bacteria in the euphotic zone (upper 150 m), compared well with the range seen by Shimada *et al.* (Shimada *et al.*, 1993). Also, our data in the Eastern North subtropical Pacific are as expected when compared with U.S. Joint Global Ocean Flux Study data from the equatorial Pacific (Binder *et al.*, 1996; Landry *et al.*, 1996).

4.1. Picoplankton community composition and carbon biomass in different marine provinces.

Picoplankton are known to be the dominant components of the planktonic community in oceanic waters. However, our results showed great variability both in picoplankton biomass and community structure among different oceanic provinces.

In the subarctic Pacific, the picoplankton community was characterized by high abundances of *Synechococcus* and picoeukaryotes and absence of *Prochlorococcus.* The picoeukaryotes are the dominant contributor to pico-sized autotrophic biomass in the North subarctic Pacific. The contribution of *Synechococcus* to photosynthetic biomass remained small compared with picoeukaryotes, though their abundance was higher. *Prochlorococcus* were not detected, although they have been reported to occur

as far north as 60°N in the North Atlantic (Buck *et al.*, 1996). Many studies reported that *Prochlorococcus* are absent from the North subarctic Pacific water of 45°N due to the lower water temperature and salinity than in the North Atlantic (Boyd and Harrison, 1999; Obayashi *et al.*, 2001; Partensky *et al.*, 1999a). In the subtropical Pacific, in contrast, the picoplankton community was characterized by abundant *Prochlorococcus* and less abundant *Synechococcus* and picoeukaryotes. *Prochlorococcus* were dominant in the total phytoplankton biomass in subtropical oceans. There were distinct decreasing trends in abundance and biomass of *Prochlorococcus* from the oligotrophic Western North Pacific Gyre to the mesotrophic Eastern North Pacific (Schlitzer, 2004), which is the opposite of the other picoplankton populations. These variations between different latitudes and along trophic gradients at the same latitude are in agreement with the intrinsic nature of the species. *Prochlorococcus* are warm water species associated with oligotrophic water while *Synechococcus*, picoeukaryotes and AAPB prefer eutrophic conditions (Jiao *et al.*, 2005; Jiao *et al.*, 2007a; Partensky *et al.*, 1999b). High-abundance values of heterotrophic bacteria occurred in the low-latitude Eastern North Pacific, but the difference in abundance between high latitude and low latitude areas was relatively small, whereas high-abundance values of AAPB occurred in the high-latitude subarctic sea, which was likely to be associated with the high chlorophyll *a* concentration there. The fact that AAPB are less influenced by low temperature compared with other bacteria may also be responsible to some extent for their distribution pattern across latitudes (Zhang and Jiao, 2007).

4.2. Habitat segregation of the picoplanktonic groups

Vertical distributions of different autotrophs are usually thought to be in agreement

due to similar control of light on their growth, but a fine differentiation was seen here between different picoplankton groups. Due to being able to utilize dim light for photosynthesis (Jiao *et al.*, 2002; Partensky *et al.*, 1999b), the maximum distribution depth of *Prochlorococcus* was deepest among all the picoautotrophs. Picoeukaryotes ranked second, and *Synechococcus* came last. AAPB, being primarily heterotrophic, are still light associated, and their distribution was never below the euphotic zone, which distinguished the AAPB from other heterotrophic bacteria (Fig. 2 and 3). In general, AAPB followed the chlorophyll *a* concentration along the depth profile (vertical profiles of chlorophyll *a* not shown). One exception was the extremely oligotrophic WNPG, where the AAPB maximum occurred at shallower depths than chlorophyll *a*. In the WNPG, since the phytoplankton did not thrive in the euphotic zone, the AAPB only maintained minimum abundance throughout the euphotic water column, with a weak maximum occurring near the surface, probably benefiting from light (Jiao *et al.*, 2007b). These observations suggest that AAPB are associated with phytoplankton. The organic matter supply from phytoplankton may be a key factor in the vertical distribution of AAPB (Zhang and Jiao, 2007).

Horizontal distributions, on the other hand, seemed to be better correlated with nutrients. *Prochlorococcus* are basically associated with oligotrophic conditions and can flourish in stratified nutrient-deplete waters (Campbell and Vaulot, 1993; Lindell and Post, 1995; Olson *et al.*, 1990), while *Synechococcus*, picoeukaryotes and heterotrophic bacteria seem to be associated more with eutrophic conditions (Fuhrman, 1999; Jiao *et al.*, 2002). Correlations analysis showed habitat segregation of the picoplanktonic groups induced by nutrients. Statistically significant positive correlations were observed between *Synechococcus* and picoeukaryotes ($r=0.98$, $p<0.01$), picoeukaryotes and bacteria($r=0.97$, $p<0.01$), *Synechococcus* and bacteria

298 (r=0.96, p<0.01), AAPB and picoeukaryotes (r=0.83, p<0.01), AAPB and
299 *Synechococcus* (r=0.82, p<0.01), and between AAPB and total bacteria (r=0.71,
300 p<0.05) (Fig. 4A-E, J). In contrast, *Prochlorococcus* showed inverse relationships
301 with other picophytoplankton and even with heterotrophic bacteria (Fig. 4F-J). Such
302 inverse relationships are also found in the Arabian Sea (Campbell *et al.*, 1998) and
303 China Seas (Jiao *et al.*, 2002). The inverse relationships between *Prochlorococcus* and
304 other picoplankton populations (Fig. 4F-J) seem to be a general feature along nutrient
305 gradients from oligotrophic to relatively eutrophic regimes (Schlitzer, 2004). In the
306 case of horizontal distribution of AAPB, since the bacterial chlorophyll *a*-based
307 phototrophic function in AAPB is a supplement to their normal organic carbon
308 respiration (Beatty, 2002; Koblizek *et al.*, 2003; Suyama *et al.*, 2002), it is thus
309 expected to make AAPB more competitive in oligotrophic environments (Beatty, 2002;
310 Kolber *et al.*, 2001; Kolber *et al.*, 2000). However, our large-scale observations
311 support the distribution pattern of higher abundance of AAPB in eutrophic water than
312 in oligotrophic water (Jiao *et al.*, 2007b; Zhang and Jiao, 2007). The strong
313 dependence of AAPB on dissolved organic carbon produced by phytoplankton may
314 limit their competition in oligotrophic oceans (Jiao *et al.*, 2007b; Zhang and Jiao,
315 2007).

316 Physical conditions are also a factor influencing the dynamics of picoplankton over
317 large spatial scales. The mixed-layer depth and the strength of the pycnocline are key
318 physical factors controlling vertical distribution of the picoplankton. A strong
319 pycnocline can behave as a barrier to the vertical transport of dissolved chemicals
320 such as nutrients. Our results showed that both the biomass of autotrophic
321 picoplankton in the upper mixed-layer and the depth attenuation were higher at
322 high-latitudes (K2) than at low-latitudes, except for the off shore station ENP4, where

the biomass and depth attenuation were highest (Fig. 3). Overall, latitude difference (mainly temperature difference) seemed to be more responsible for changes of picoplankton community structure while nutrient availability was more important for picoplankton biomass differences.

4.3. Potential contribution of the picoplankton to carbon cycling in the upper ocean

Pico-sized phytoplankton biomass could be significant in carbon export from the surface ocean. Their potential export pathways include aggregation and incorporation into settling detritus, and indirect export through consumption of picoplankton aggregates by organisms at higher trophic levels (Barber, 2007; Buesseler *et al.*, 2007; Richardson and Jackson, 2007). The main contributors to autotrophic picoplankton biomass were different at the sites investigated. *Prochlorococcus* contributed most carbon biomass to the total autotrophic picoplankton biomass in the oligotrophic subtropical Pacific, while picophytoplankton biomass was dominated by picoeukaryotes at K2 and was co-dominated by *Synechococcus* and picoeukaryotes at station ENP4 (Fig. 5B). Biomass ratios of autotrophic to heterotrophic picoplankton were 2-2.5 at K2 and ENP4 and ~1 at WNPG1-2 and ENP1-3, showing significant difference among the different provinces ($p<0.01$). This revealed that within the picoplankton community, autotrophic picoplankton make a higher contribution to total picoplankton biomass in mesotrophic or relatively eutrophic areas, while heterotrophic bacteria become more important in oligotrophic oceans as they contribute more to carbon cycling through the “microbial loop” (Azam *et al.*, 1983).

Although accounting for only a small proportion of the total heterotrophic bacteria in terms of abundance, the AAPB are an important functional member of the

348 community and may play a unique role in carbon cycling in the upper ocean
349 ecosystem. Their ability to supplement or substitute respiration with the light-driven
350 generation of ATP and reductants for carbon anabolism preserves the existing organic
351 carbon (Kolber *et al.*, 2001). In terms of carbon export, the cell volume of AAPB on
352 average is usually 2-4 times greater than the other heterotrophic bacteria (present
353 measurements) (Sieracki *et al.*, 2006; Yurkov and Beatty, 1998a). It is therefore
354 speculated that AAPB cells are easily grazed (Sieracki *et al.*, 2006) and can settle out
355 of the euphotic zone (Barber, 2007; Richardson and Jackson, 2007) forming vertical
356 flux. Furthermore, their rather rapid growth (Koblizek *et al.*, 2007; Koblizek *et al.*,
357 2005) fuels the flux of carbon export more than their abundance alone would predict.
358 In the present study, the biomass contribution of AAPB was significantly higher at K2
359 than other stations ($p<0.01$) (Fig. 5C). Contribution of the AAPB to the total bacterial
360 carbon biomass increased significantly with increasing nutrient conditions, from the
361 Western North Pacific subtropical Gyre to the Eastern North Pacific, to the eastern
362 Pacific off shore water and to the subarctic sea (Schlitzer, 2004) (Fig. 5C). These
363 results implied that the contribution of AAPB to the oceanic sink of carbon may be
364 more important in high latitude areas than in low latitude areas.

365 In the traditional food web, large members of the phytoplankton like diatoms are
366 believed to control carbon flux from the upper ocean, especially when they form
367 blooms. However, picoplankton can also be an important source of organic carbon for
368 large zooplankton such as copepods and for the particulate organic carbon pool that
369 fuels the flux of particles sinking to the deep ocean (Barber, 2007; Buesseler *et al.*,
370 2007; Richardson and Jackson, 2007). The contribution of primary producers to
371 carbon export from the surface layer of the ocean is reported to be at rates
372 proportional to those of their production (Barber, 2007; Richardson and Jackson,

2007). As known from other studies in the VERTIGO Project, K2 is a site of high diatom biomass (Buesseler *et al.*, 2008). Oligotrophic North Pacific regions in contrast have low nano- or micro-phytoplankton biomass, but high pico-phytoplankton biomass (Buesseler *et al.*, 2008; Shimada *et al.*, 1993). Therefore, picoplankton could be an important source of new organic carbon for upper trophic level organisms and for detritus production, and thus the export flux from the surface layer to the deep sea, especially in the oligotrophic subtropical Pacific.

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553 **Table 1 Physical and chemical conditions at the sampling sites[a]**

554

	K2	WNPG1	WNPG2	ENP1	ENP2	ENP3	ENP4
Surface water temperature (ºC)	10.3±0.5	27.7	28.2	27.5	28.2	28.1	28.5±0.03
Surface water salinity (‰)	32.9±0.01	34.4	35	34.4	34.1	34.2	34.1
Depth of mixed layer (m)	25	100-125	100-125	75	75	50-75	30
$Chlorophyll_{max}$ depth (m)	50	125	125	75	75	50-75	30
Euphotic zone (0.1% light) (m)	50	150	150	125	125	125	75
Surface chlorophyll *a* (mg m^{-3})	0.35±0.05	0.03	0.03	0.12	0.11	0.10	0.13±0.01
Chlorophyll *a* averaged over upper 200m (mg m^{-3})	0.27±0.04	0.08	0.08	0.10	0.11	0.10	0.12±0.005

555 a. K2: subarctic sea area; WNPG: the Western North Pacific subtropical Gyre; ENP:

556 the Eastern North subtropical Pacific.

Table 2 Cell abundance and carbon biomass of picoplankton at different locations

Cell abundance (cells ml^{-1})[a]	K2 [b]	WNPG1	WNPG2	ENP1	ENP2	ENP3	ENP4 [b]
Prochlorococcus	ND [c]	85500	100800	67600	70400	61400	60300±13500
Synechococcus	13900±1400	1300	1700	2100	3700	3200	41000±15700
Picoeukaryotes	5200±600	570	730	830	1700	1400	6300±1500
Heterotrophic bacteria	382800±38500	249400	283500	318200	358900	329300	528000±98800
AAPB	14000±900	1700	2100	2900	3200	3200	7900±2900
Biomass (mg C m^{-3})[a]							
Prochlorococcus	ND [c]	4.53	5.34	3.58	3.73	3.25	3.20±0.71
Synechococcus	3.47±0.36	0.34	0.44	0.52	0.93	0.80	10.25±3.94
Picoeukaryotes	10.86±1.20	1.19	1.53	1.74	3.64	3.01	13.14±3.08
Heterotrophic bacteria	7.66±0.77	4.99	5.67	6.36	7.18	6.59	10.56±1.98
AAPB (mg C m^{-3})	1.01±0.06	0.12	0.15	0.21	0.23	0.23	0.57±0.21
Autotrophic picoplankton C / heterotrophic bacterial C	1 : 0.53	1 : 0.82	1 : 0.78	1 : 1.09	1 : 0.87	1 : 0.93	1 : 0.40

a. Data were depth weighted averages in corresponding euphotic zone (see Table 1).

b. Values of standard deviation (SD) were calculated from two deployments (four CTD casts for each deployment) at K2, and from four deployments (one CTD cast each deployment) at ENP4.

c. ND = not detected.

Figures Legends:

Fig. 1 Location of the sampling stations (crosses) in the North Pacific Ocean. The background chlorophyll *a* remote image (Aqua-MODIS) of August 2005 was downloaded from the website (http://oceancolor.gsfc.nasa.gov/). Chlorophyll *a* scale shown on right in mg m^{-3}.

Fig. 2 Depth profiles of picoplankton abundance in the subarctic area (K2), the Western North Pacific subtropical Gyre (WNPG1-2) and the Eastern North subtropical Pacific (ENP1-4). Error bars indicate standard deviation. Values of SD were calculated from the data of samples from four CTD casts each deployment at K2 and at ENP4. Note different X-axes are used. Pro.: *Prochlorococcus*; Syn.: *Synechococcus*; Euk.: picoeukaryotes; Total Bact.: total heterotrophic bacteria.

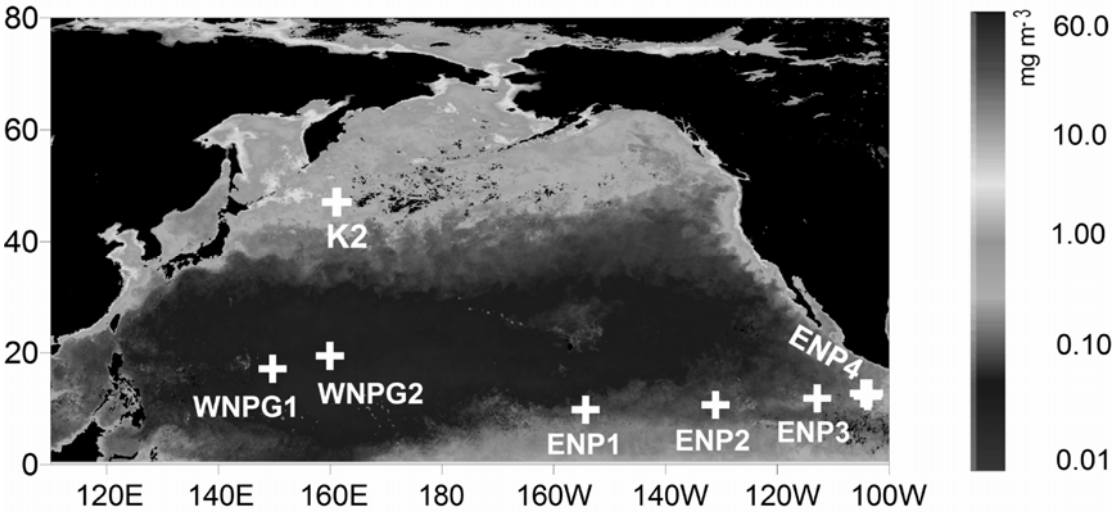
Fig. 3 Depth profiles of picoplankton carbon biomass (X-axis: autotrophic-left and heterotrophic-right of zero) in the subarctic area (K2), the Western North Pacific subtropical Gyre (WNPG1-2) and the Eastern North subtropical Pacific (ENP1-4). In order to obtain complete vertical profiles of picoplankton carbon biomass at K2, data from 50-200m (shaded) were fitted by including the results of a near-K2 station at 50ºN 163ºE during our Bering Sea cruise in July 2003. Pro.: *Prochlorococcus*; Syn.: *Synechococcus*; Euk.: picoeukaryotes. non-AAPB: other heterotrophic bacteria excluding AAPB.

Fig. 4 Relationships between different groups of picoplankton. A, *Synechococcus* vs. picoeukaryotes; B, picoeukaryotes vs. bacteria; C, *Synechococcus* vs. bacteria; D,

590 AAPB vs. picoeukaryotes; E, AAPB vs. *Synechococcus*; F, bacteria vs.
591 *Prochlorococcus*; G, picoeukaryotes vs. *Prochlorococcus*; H, *Synechococcus* vs.
592 *Prochlorococcus*; I, AAPB vs. *Prochlorococcus*; J, AAPB vs. bacteria. Abundance
593 data are depth-weighted averages over the euphotic zone. Pro.: *Prochlorococcus*; Syn.:
594 *Synechococcus*; Euk.: picoeukaryotes; Total Bact.: total heterotrophic bacteria.
595
596 **Fig. 5** Contribution of different groups of picoplankton to carbon biomass in the
597 subarctic area (K2), the Western North Pacific subtropical Gyre (WNPG1-2) and the
598 Eastern North subtropical Pacific (ENP1-4). A, total heterotrophic bacteria (Total
599 Bact.) vs. pico-sized phytoplankton (picophyto.); B, picoeukaryotes (Euk.) vs.
600 *Synechococcus* (Syn.) vs. *Prochlorococcus* (Pro.); C, AAPB vs. non-AAPB (other
601 heterotrophic bacteria excluding AAPB). Values are % of carbon biomass of
602 picoplankton calculated by depth-weighted average over euphotic zone.

603 **Figure 1**

604



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606 **Figure 2**

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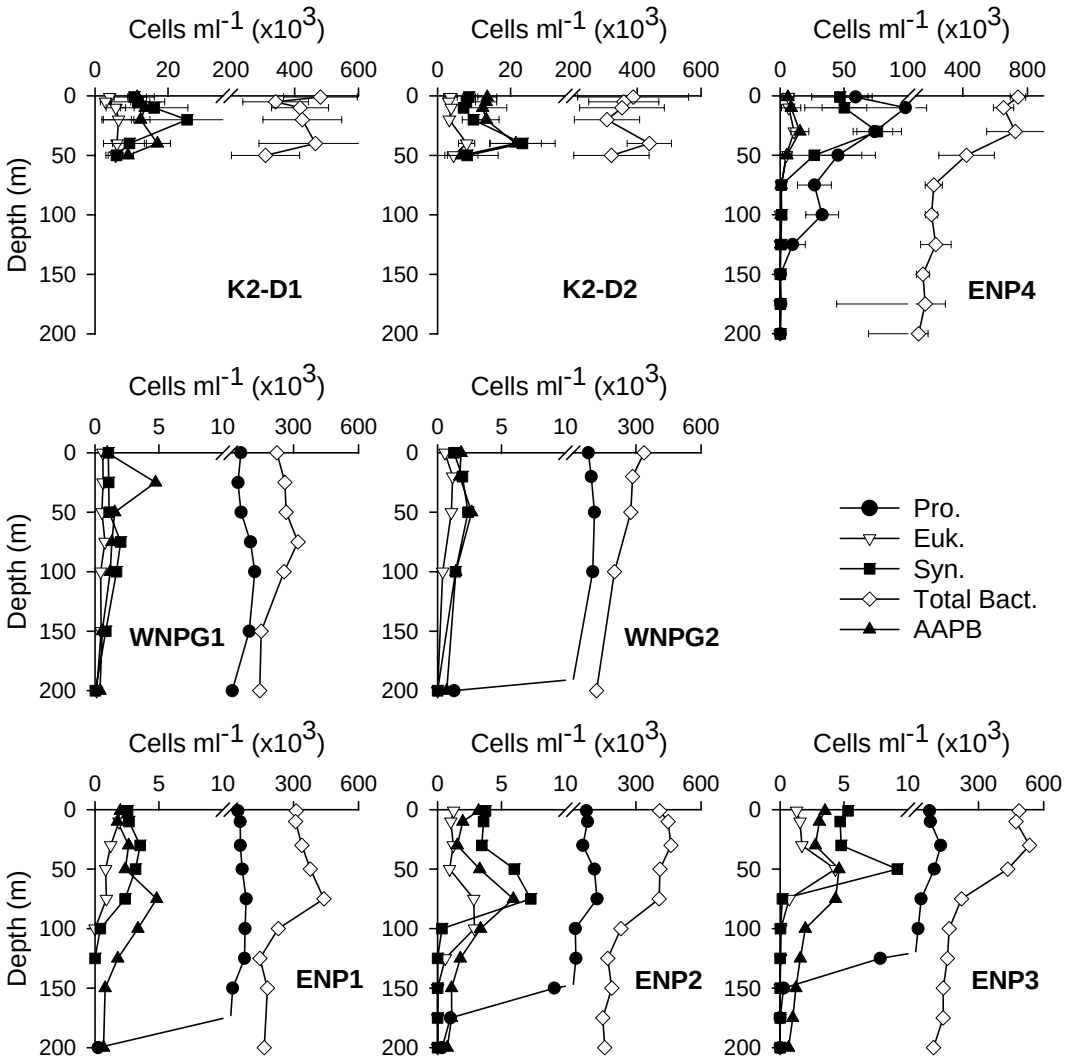

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609 **Figure 3**

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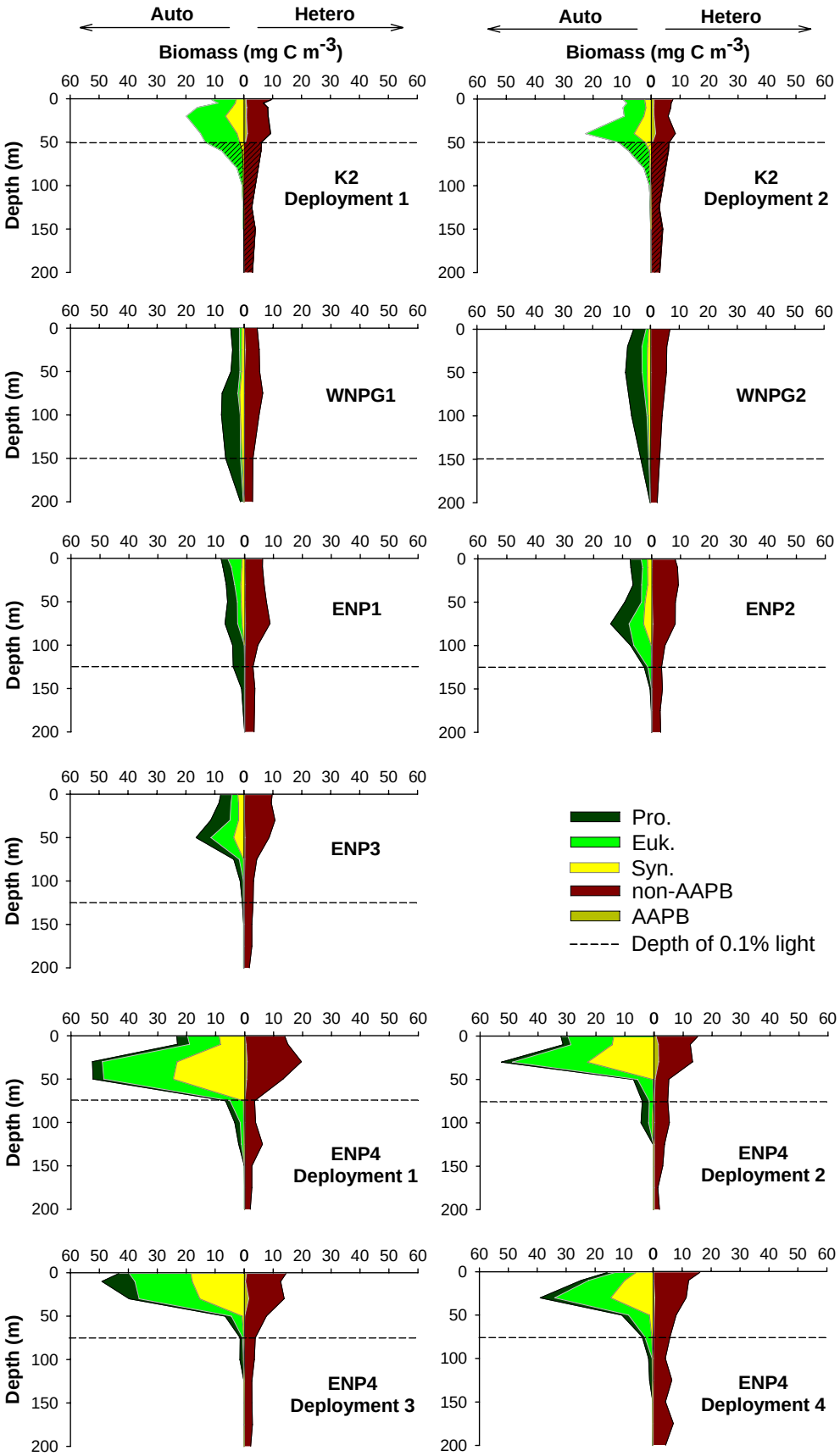

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612 **Figure 4**

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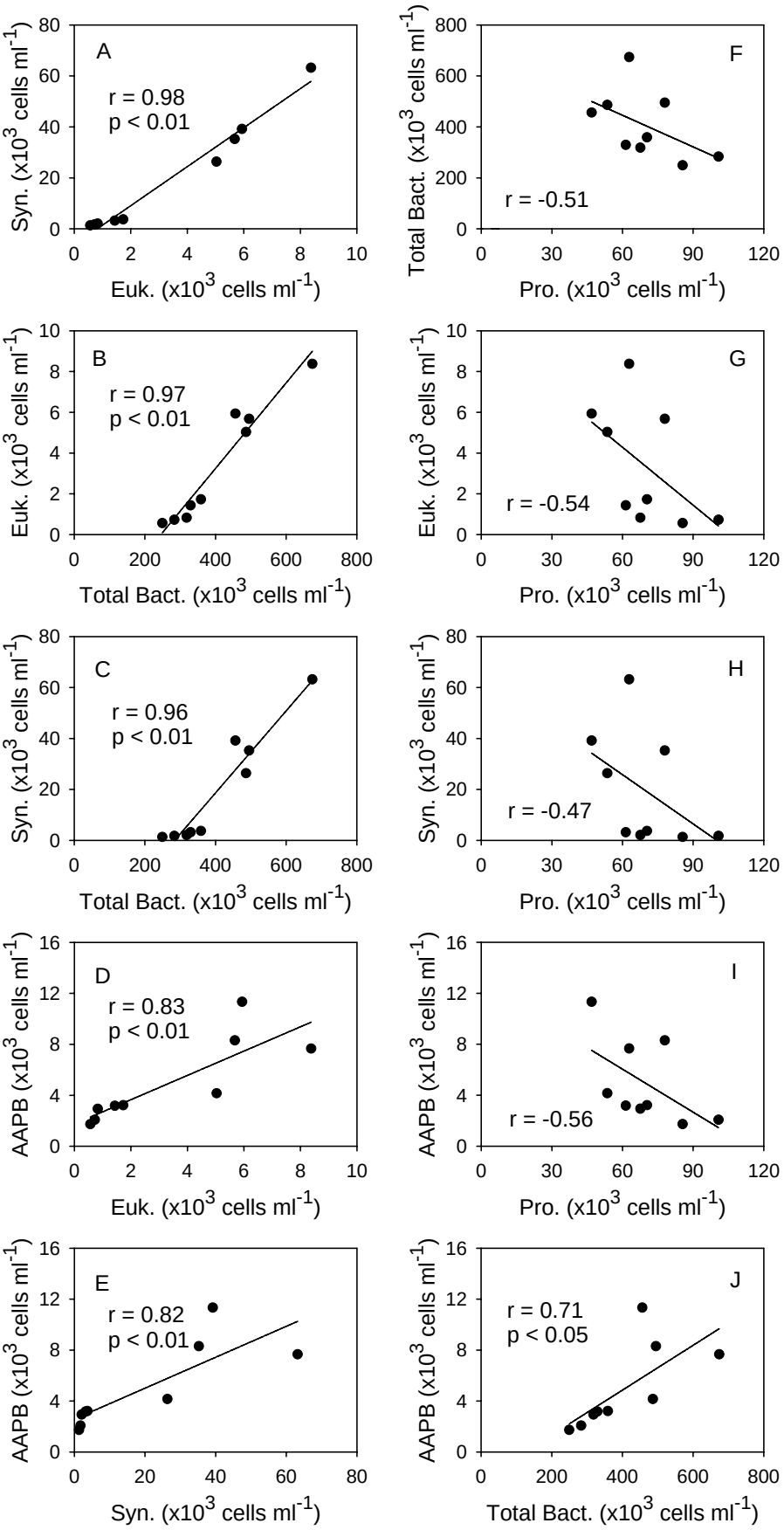

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615 **Figure 5**

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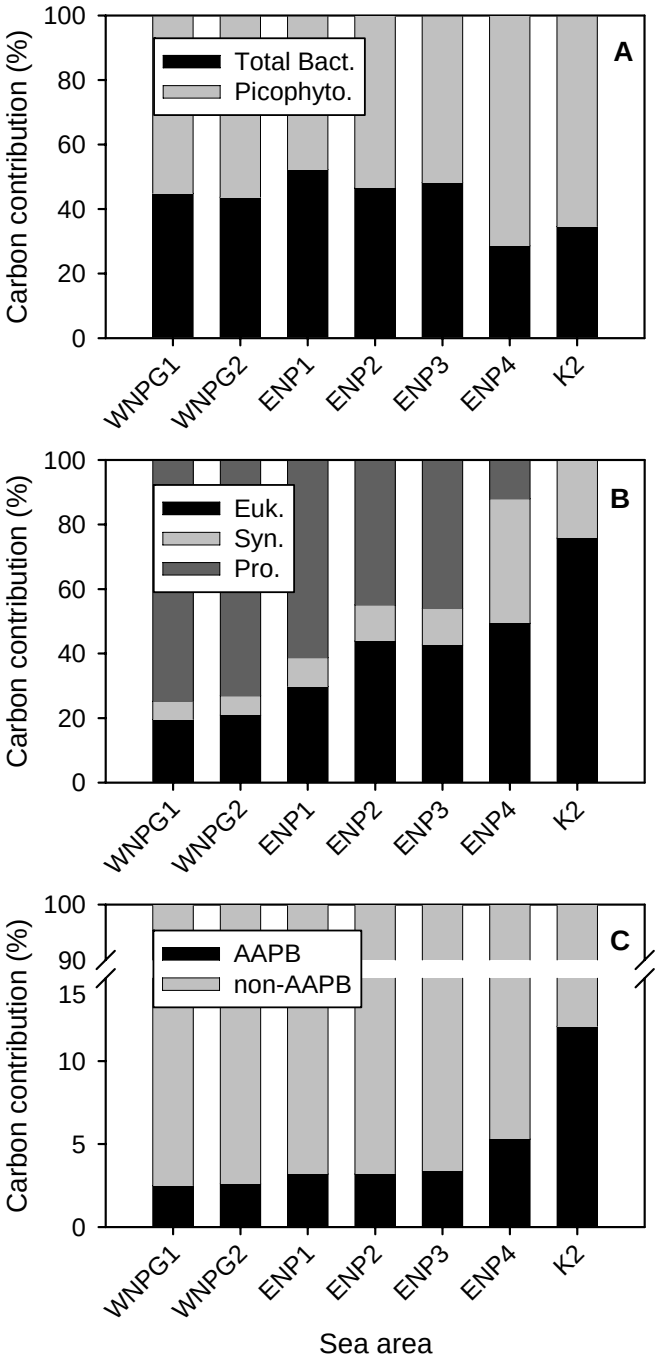

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