SUPPLEMENTARY MATERIAL

Methods

Incubations were performed in 2L duplicate polycarbonate bottles (Nalgene) that were suspended inside a large concrete tank with running water that controlled temperature. The experiment lasted 3 days. Nutrients were added to final concentrations of 10 μM C (glucose), 1.6 μM N (NH_4Cl) and 0.1 μM P (KH_2PO_4) to yield the following treatments: control (with no additions), +P, +N, +PC, +NP +NPC.

Bacterial heterotrophic activity was determined in all mesocosms every day by incorporation of tritium-labeled leucine (Kirchman et al., 1985) using the centrifugation procedure. Triplicate samples and one prefixed control sample were incubated with ^{3}H-Leucine (4.27 TBq mmol-1, Perkin Elmer, Boston, USA) at a final concentration of 60 nM. Incubation was performed in the dark at *in situ* temperature for 1 h and stopped with 5% TCA, final concentration. The samples were then centrifuged at 16000 x g for 10 min before removal of the supernatant., were washed twice by adding 5% TCA, vortexed, centrifuged and the supernatant removed. Counting cocktail (Ecoscint A, National Diagnostics, Atlanta, USA) was added and the incorporation of radioactive leucine measured by liquid scintillation counting.

Turnover time of phosphate (Pi) was estimated using $^{33}PO_4^{3-}$. $H_3{}^{33}PO_4$ (40-158Ci mg^{-1}; Perkin Elmer) was diluted in distilled water and 15-μL aliquots were added to duplicate 9-mL subsamples to give final concentrations ranging between 25 and 108 pM. Duplicate killed controls were included with each set of samples. Killed controls were amended with paraformaldehyde (2% final concentration) 30 min before the addition of the isotopic tracer. Incubations were done in 15-mL Falcon tubes at room temperature and subdued light. The duration of each incubation varied depending on the expected turnover time, ranging from 20 min to 2 h. Incubations were terminated by the addition of paraformaldehyde (2% final concentration) and filtered, within 30 min, onto a 0.2 μm polycarbonate filter, which was placed on top of a Whatman (GF/C) glass fiber filter saturated with 100 mmol L^{-1} KH_2PO_4. To stop incubations, fixation was chosen over cold-chase addition of cold PO_4^{3-} to be consistent with the methodology employed during the MARFISH analyses (see below). Fixation has an experimental limitation, which is that some of the accumulated ^{33}P could leak out of fixed cells after the cell membranes become compromised. Talarmin et al. (2011) recently reported that up to 40% of the ^{33}Pi label is lost from fixed heterotrophic bacterial cells immediately after fixation and Casey et al. (2009) estimated that ~25% of the isotope in Pi incubations could leak out from the cells within 24h. To minimize this leakage, samples were filtered within 1h after stopping the incubation. After filtration, the filters were rinsed twice with sterile Milli-Q water and transferred to scintillation vials with 1 mL Ultima Gold scintillation cocktail. Aliquots (50 μL) from the subsamples incubated with $^{33}PO_4^{3-}$ were transferred directly to scintillation vials and mixed with 1 mL scintillation cocktail to measure the total added radioactivity. Samples were radioassayed in a Packard Tri-Carb 4000 scintillation counter. Turnover times were calculated using the equation $T = t / [-ln (1-R)]$, where t = incubation time and R= consumed fraction of added tracer (Thingstad et al., 1993). Killed controls were subtracted prior to the calculations.

For MARFISH analyses 30-mL subsamples were spiked with ^{3}H-leucine (Perkin Elmer) to yield 0.5 nM. The samples were incubated for 2.5 h. One simple was killed with paraformaldehyde before the addition of the radiolabeled substrate and was used as a control. At the end of the incubation, samples were fixed with paraformaldehyde, allowed to sit in the dark for at least one hour, and then portions of 5-10-mL were filtered onto three different 0.2 μm polycarbonate filters. Filters were washed twice with sterile Milli-Q water and frozen at -80°C until processing in the lab. Filters were then hybridized following the CARD-FISH protocol (Pernthaler et al., 2002) to identify the different bacterial groups. After thawing, the filters were dipped in 0.1% agarose, dried at 37°C, and then dehydrated with 95% ethanol. This allowed attachment of the cells to the filters. Then, cell walls were permeabilized with lysozyme (1 h) and achromopeptidase (30 min) at 37°C. Filters were cut into multiple pieces and hybridized with one of the following horseradish peroxidase (HRP)-labeled probes: EUB338 I-II and –III (targets most *Eubacteria*, Daims et al., 1999), GAM42a together with its unlabeled competitor probe (targets most *Gammaproteobacteria*, Manz et al., 1992), CF319a (targets many members of the *Bacteroidetes* group, Manz et al., 1996), ROS537 (targets members of the *Alphaproteobacteria Roseobacter-Sulfitobacter-Silicibacter* group, Eilers et al., 2000), SAR11-441R (targets the *Alphaproteobacteria* SAR11, Morris et al., 2002), ALT1413 (targets *Alteromonadaceae*, Eilers et al., 2000), or NOR5-730 (targets the NOR5/OM60 clade, Eilers et al., 2000). Specific hybridization conditions were established by addition of formamide to the hybridization buffers (45% formamide for the SAR11 probe, 50% for the NOR5-730 probe, 60% for the ALT1413 probe, and 55% for the other probes). Hybridization was performed overnight at 35°C. For amplification, we used tyramide labeled with Alexa 488. After processing, a small portion of the filter was cut and stained with 4',6-diamidino-2-phenylindole (DAPI, final concentration 1 μg mL^{-1}) to quantify the abundance of the different phylogenetic groups in relation to total prokaryotic counts. The rest of the filter was glued onto a glass slide and subsequently processed for microautoradiography as described in detail in Alonso-Sáez and Gasol (2007), which is a modification of the protocol described by Alonso and Pernthaler (2005). Exposure times were determined empirically by following changes in number of cells taking up the substrate over time. Optimal exposure times were selected once the number of cells taking up the substrate reached a plateau but accumulation of silver grains still allowed visualization of the cells associated to them. Cells were counted in an Olympus BX61 epifluorescence microscope. Cells touching or overlapping silver grains after developing of the emulsion were considered as active cells or MAR+ cells. For abundance of probe-positive cells, between 500 and 1000 DAPI-positive cells were counted manually in a minimum of 10 fields. Killed controls were evaluated with the probe EUB338 I-II and –III. The proportion of labeled cells in the killed controls was 2%. This proportion was not subtracted from the percent of cells taking up ^{3}H-leucine in the live incubations.

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SUPPLEMENTARY FIGURES

Figure S1.- RESPONSE OF THE TURNOVER TIME OF PHOSPHATE TO NUTRIENT ADDITIONS IN P-STARVED MEDITERRANEAN WATERS

To assess the bulk community response to nutrient additions we first estimated the turnover time of phosphate (Pi) to see how fast this nutrient was utilized in these P-starved waters, and how the different treatments affected its utilization.

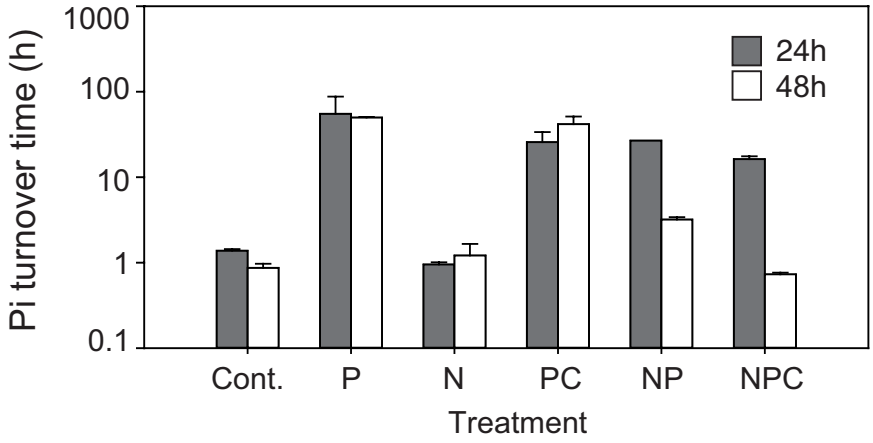

Figure S1 legend. *Phosphate turnover time in the different treatments on day 1 and day 2 of the experiment. Samples were amended with phosphate (P), ammonia (N), and organic carbon (C), or with combinations of these nutrients (PC, NP, NPC). Cont.: control treatment (no amendments). Each data point represents the average of the two replicates. Error bars represent the standard deviation.*

The turnover time in the control treatment was ~ 1 h, which means that all the bioavailable pool of Pi would be used in the timespan of 1 h if the supply of Pi stopped. All the treatments where P was added resulted in a notable increase in the turnover time of Pi within the first 24 h of the experiment. However, the Pi turnover time in the NP and NPC treatments after 48 h returned to values close to those observed in the control. These results imply that the Pi added could not be entirely used in the P treatment due to the lack of enough N and C, which is supported by the observation that heterotrophic bacteria in these waters accumulated polyphosphates when Pi was added alone (Sebastián et al., 2012).

Sebastián, M., Pitta, P., González, J.M., Thingstad, T.F., and Gasol, J.M. (2012) Bacterioplankton groups involved in the uptake of phosphate and dissolved organic phosphorus in a mesocosm experiment with P-starved Mediterranean waters. Environmental Microbiology 14: 2334-2347

Figure S2.- RESPONSE OF THE COMMUNITY COMPOSITION TO THE NUTRIENT ADDITIONS

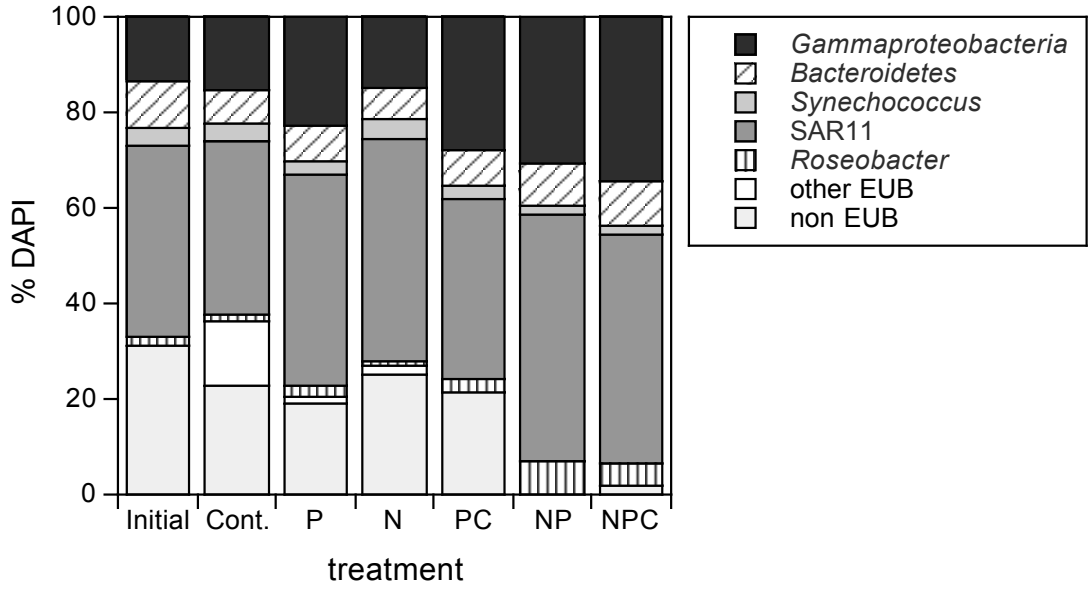

Figure S2 legend*. Community composition before the start of the experiment and in the different treatments at the moment the MARFISH analyses were performed. Data are presented as percent contribution of the probe-identified groups to total number of prokaryotes (DAPI counts). Treatments correspond to the following amendments: phosphate (P), ammonia (N), and organic carbon (C), or combinations of them (PC, NP, NPC). Initial: before the start of the experiment. Cont.: control treatment (no amendments).*

Figure S3.- EFFECT OF NUTRIENT ADDITIONS IN THE TOTAL ABUNDANCE OF ACTIVE CELLS WITHIN EACH PROBE-IDENTIFIED GROUP

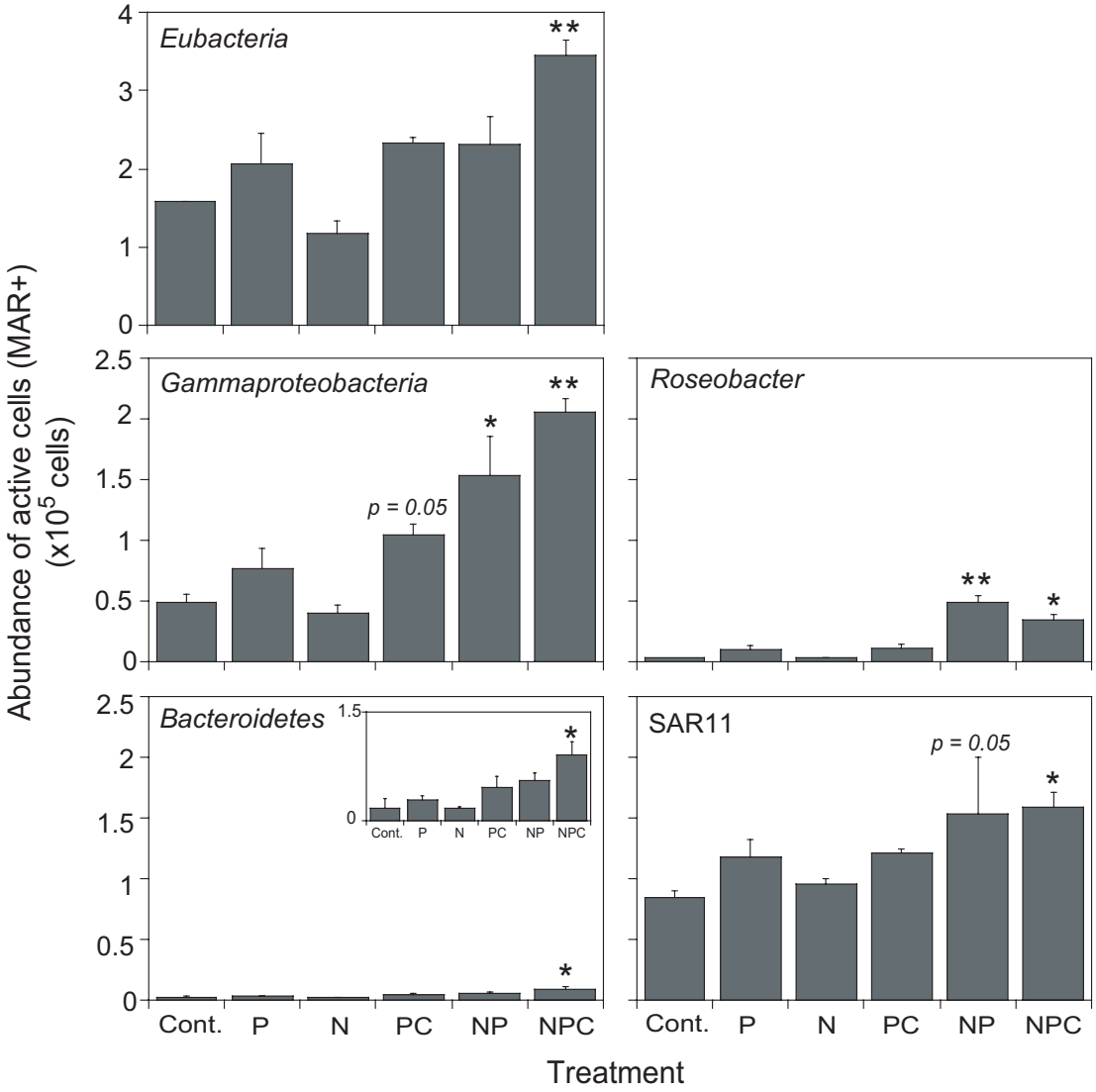

***Figure S3 legend**. Abundance of cells active in ^{3}H-leucine incorporation belonging to each probe-identified group. Labels as defined in Figure S1. Asterisks denote significant differences in relation to the control (*: p< 0.05, **: p< 0.001). Insert in the left lower panel is an expanded view of the abundance of cells in the Bacteroidetes lineage. Note the different scale in the Y-Axis for Eubacteria (upper panel).*

Figure S4.- RELATIONSHIP BETWEEN BACTERIAL ACTIVITY AND NUMBER OF ACTIVE CELLS

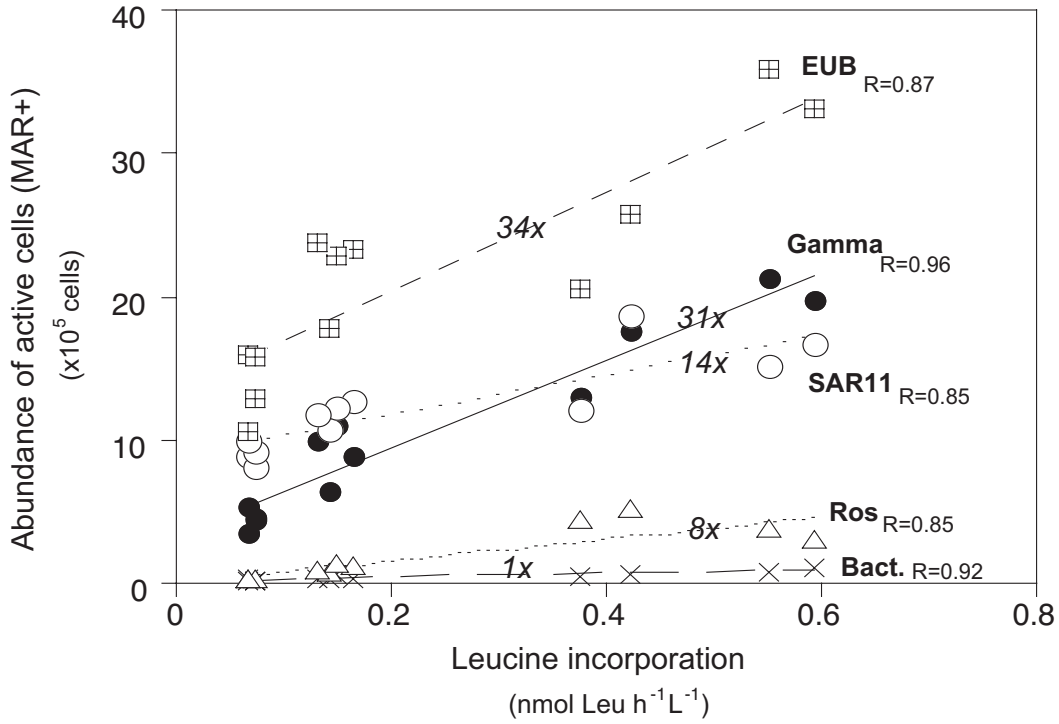

Figure S4 legend. *Relationships between the total numbers of active cells within each probe-identified group and the bulk leucine incorporation measured for each of the samples. EUB: Eubacteria, Gamma: Gammaproteobacteria, Ros: Roseobacter, Bact: Bacteroidetes. Numbers in italics represent the slopes of the linear fit. Note the similarity in the slopes of the Gammaproteobacteria and total bacteria (Eubacteria) relationships.*

Figure S6.- *GAMMAPROTEOBACTERIA* COMPOSITION

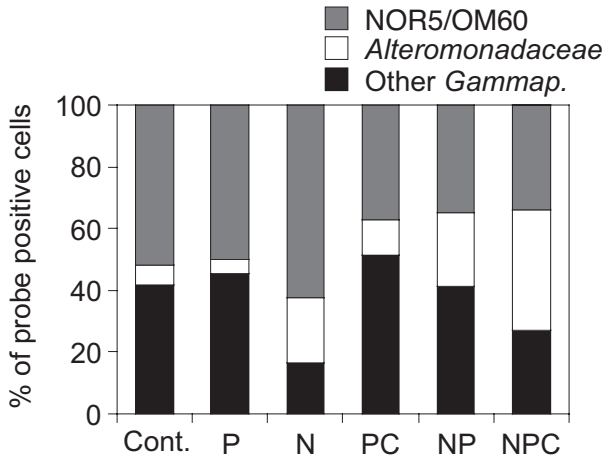

Figure S6 legend*. Composition of the gammaproteobacterial population in the control and nutrient amended treatments.*