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Supplement of

Heterotrophic bacterial production and metabolic balance during the VAHINE mesocosm experiment in the New Caledonia lagoon

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Material and Methods

Specific leucine assimilation assessment using cell sorting

On four occasions (day 15, M1, 6 m; day 19, M1, 6 m; day 21, lagoon waters, 1 m; day 23, M3, 1 m), additional seawater samples were incubated with ^{3}H leucine for further cell sorting of phytoplankton and heterotrophic bacteria: five polypropylene (Nunc) tubes (3 live samples, 2 killed controls) were filled with 4.5 mL of seawater and incubated with pure ^{3}H leucine (10 nM final conc.) for 1 h in the on-deck incubator as for bulk samples (section 2.3). Incubations were stopped by addition of 0.5 mL of 20 %, 0.2 µm-filtered formalin, stored in the fridge for 15 minutes, and then stored in liquid N_2 until sorting. Two additional non-incubated tubes (but fixed and stored in the same conditions) were used for total counts. Sorting of the radiolabeled samples were performed with on a BD Influx^{TM} Mariner (BD Biosciences, San Jose, CA) high speed cell sorter equipped with three laser lines: 488 nm (Sapphire, Coherent), 561 nm (Jive, Cobolt) and 355 nm (Xcyte, JDSU). All data were collected in log scale, stored in list mode files and analyzed in real time for sorting using the BD FACSortware software (BD Biosciences), or analyzed a posteriori using FlowJo v7.6.5 software (Tree Star). A solution of 0.5x PBS, 0.2 µm filtered, was used as sheath fluid. For heterotrophic prokaryote analyses and sorting, (using a drop purity sort mode), cells were stained with SYBR Green II as described above in section 2.2. Cells were characterized by two main optical signals collected from the 488 nm laser: side scatter (SSC) and green fluorescence ($530/40_{488\ nm}$), related to nucleic acid staining. Based on these criteria, low nucleic acid (LNA), high nucleic acid (HNA), and HNA with high SSC (Hi-HNA) groups were determined (Fig. 2) and sorted into different tubes. Like for determination of LNA and HNA abundances (section 2.2), phototrophic cells were excluded thanks to their red fluorescence. To analyze and sort the photosynthetic phytoplankton cells, the three laser lines were used. Red fluorescence ($630LP_{488\ nm}$) related to chlorophyll *a* content, was used as trigger signal. Phytoplankton cells were characterised by three other optical signals: forward scatter (FSC) related to cell size, side scatter (SSC), and the orange fluorescence ($580/30_{488\ nm}$) related to phycoerythrin. In addition, the chlorophyll *a* red fluorescence was collected from the 355 nm and 561 nm excitation ($630LP_{355nm}$ and $630LP_{561nm}$). The cytogram red fluorescence (induced by the 561 nm laser) vs orange fluorescence induced by the 488 nm laser evidenced two different subgroups of *Synechococcus* (one with Low and one with a High Orange fluorescence intensity, referred to as LO-SYN and HO-SYN, respectively, Fig. 2). Thus, four populations < 2 µm were optically resolved and sorted simultaneously, directly into separate 2 mL

Eppendorf centrifuge tubes, using the drop purity sort mode: *Prochlorococcus* (PRO), LO-SYN and HO-SYN, and pico-eukaryotes (PE).

Variable numbers of cells were sorted per sample depending on experiment and cell type, to achieve sufficient signal and a good compromise with the volume available. The phytoplankton collected cells ranged from 50 000-279 000 for the LO-SYN and HO-SYN groups, 16 000- 41 000 for the PE, to 1 600-10 000 for the PRO group. From the 1 mL SYBR Green II stained aliquot, the three heterotrophic prokaryote groups were simultaneously collected into separate 2 mL centrifuge plastic tubes, collecting a range of 100 000-250 000 cells for LNA and HNA groups, and 15000-53000 cells for Hi HNA group. After sorting, 1.5 mL of 5 % TCA was added into each tube and processed as for BP measurements. Bulk activities were realized in triplicate by subsampling directly 1.0 to 1.5 mL of samples from the 5 mL tubes. In these tubes, 55 % TCA was added to give a final 5 % TCA concentration and the three series of centrifugations were run as for the BP measurements. Formalin-killed samples were also sorted in order to estimate blank values for each group. Dpm in the killed control of a given group were subtracted from dpm in the corresponding incubated sample. Blank values were independent of the number of sorted cells (on average 27 ± 9 dpm). We checked that a linear increase of the dpm signal occurred with the number of sorted cells. The coefficient of variation between triplicate sorts ranged 1-5 % when dpm signal were > 1000 dpm, but these values increased up to 30 % when the dpm signal were < 100 dpm due to methodological limitations (limited volume available or lower activities). For this reason we considered below detection limits all sorts where dpm values were less than twice the corresponding blank value. The radioactivity per cell was calculated and expressed in C units (10^{-21} mol leu cell^{-1} h^{-1}). It was multiplied by the abundance of cells mL^{-1} within in sorted region to obtain the volumetric incorporation rate of each group, and the relative population activity was calculated as the population fraction of the bulk (i.e. total community) activity. Considering a drop frequency set at 98500 per second and average sorting rates of 891 cells per second for heterotrophic bacteria, the probability of free bacteria being sorted simultaneously with an autotrophic cell in the same drop, leading to overestimation of leucine assimilation rate per cell for autotrophic cells, was 0.9 % and thus considered negligible. The effect of a long storage of the samples before cell sorting was checked by counting abundance of cells mL^{-1} directly on the Influx. For this, we used the additional non labeled tubes, ran using similar procedure (sample preparation with control beads, and flow rate determination) as described in section 2.2., except that we counted also Hi-HNA cells and the two categories of *Synechococcus*.

Figure S1. Evolution of: a) bacterial production and b) alkaline phosphatase in the mesocosm M1 at the three depths sampled. For each day 1 m, 6 m and 12 m are presented from left to right. For the BP plot error bars are standard deviations within triplicate measurements. For phosphatase activity, error bars are the standard errors of the slope of the linear regression MUF production as a function of time. The vertical bar between day 4 and 5 indicates DIP fertilization.

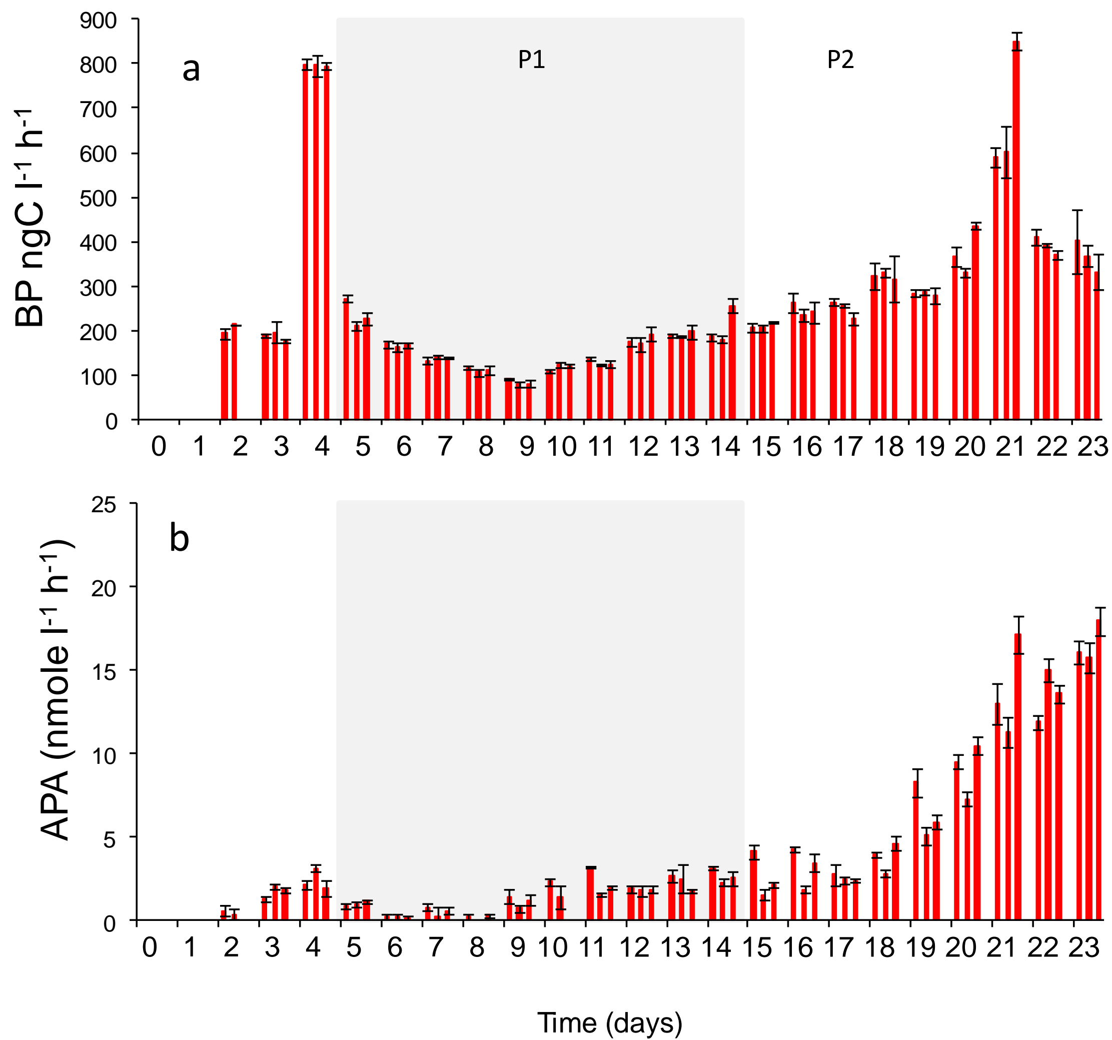

Table S1 : C budget. Time integrated measurements (average of the three mesocosms); sd-meso: standard deviation associated to the variability within the three mesocosms; sd-CF: standard deviation reflecting the range of conversion factors or analytical methods and assumptions. The errors considered for estimating sd-CF are discussed in the text: 30 % for GPP, 25 % for BP and 10 % for Cexp, DOC and POC. The sd in italics are computed assuming general laws of error propagation.

	Time integrated measurements	sd -meso	sd -CF
	µMC	µMC	µMC
GPP	38.1	11.2	11.4
Cexp	6.4	2.1	0.6
POC	3.1	5.4	0.3
DOC	1.3	3.6	0.1
BP	10.4	0.6	2.6
resp 1=CR	27.3	*11.6*	*11.5*
resp 2=CR/2	13.7	*5.8*	*5.7*
	%	*%*	*%*
BGE1	27 %	*9 %*	*10 %*
BGE2	43 %	*11%*	*12 %*