

1 Dear Referees,

2
3 First, we would like to thank you very much for your constructive comments. We are pleased
4 to provide a revised version of our manuscript "*Biogeochemical and biological impacts of*
5 *diazotroph blooms in a Low Nutrient Low Chlorophyll ecosystem: synthesis from the VAHINE*
6 *mesocosm experiment (New Caledonia)*". We made our best to take into consideration all
7 comments and suggestions. Comments and questions are in regular font with our replies
8 below in bold font. The marked-up manuscript is also provided below.

9
10 Sophie Bonnet on behalf of co authors
11
12
13

14 Referee #1

15
16 SPECIFIC COMMENTS

17 p.16: 19-20 – Authors argue that detritus and DON ... "likely provided" the balance of
18 bacterial N demand unaccounted by DDN since concentrations of these two components
19 "decreased during the 23 days of the experiment". A couple of rapid calculations can easily
20 dispel this doubt providing a better view of relative magnitudes and revealing gaps in this
21 budget if at all.
22

23 **The sentence has been modified as follows: "Calculations based on C:N molar ratios show**
24 **that N₂ fixation may have provided ~30 % of the N demand of the N-limited bacteria**
25 **during P2 (compared to ~20 % during P1), the rest (representing 0.6-0.7 μmol L⁻¹) was likely**
26 **provided by detritus and DON (Van Wambeke et al., 2015), which concentrations**
27 **decreased by ~0.9 μmol L⁻¹ during the 23 days (Berthelot et al., 2015b)."**

28
29 p. 17:7-28 - The review of the role of *Trichodesmium* in N export, while pertinent to the
30 discussion, is beyond the scope of the mesocosm experiment and should thus be
31 abbreviated considerably
32

33 **A *Trichodesmium* bloom occurred during the VAHINE experiment, albeit outside the**
34 **mesocosms. Nevertheless, this bloom has been characterized and results are presented in**
35 **Spungin et al. (2016) in the present special issue. We strongly believe that these results are**
36 **worth considering in the present synthesis article. Nevertheless, we considerably reduced**
37 **this section as follows:**

38 **"Similar experiments (¹⁵N₂ labelling, flow cytometry cell sorting and nanoSIMS) performed**
39 **on three naturally-occurring *Trichodesmium* spp. blooms in the southwestern Pacific**
40 **illustrated that DDN was predominantly transferred to diatoms (Bonnet et al., Accepted).**
41 **These results indicate that the extensive oceanic blooms of *Trichodesmium* spp. can**
42 **contribute to a subsequent indirect yet large downward flux of organic matter by**
43 **promoting large cells growth (e.g., diatoms and dinoflagellates) characterized by efficient**
44 **export rates (Nelson et al., 1995, Bonnet et al., Accepted; Devassy et al., 1979; Lenés et al.,**
45 **2001).**

46 **Direct export flux of *Trichodesmium* spp. blooms may also occur in cases where rapid (< 2**
47 **d) bloom mortality occurs via a programmed cell death (PCD) (Berman-Frank et al., 2004;**

48 Berman-Frank et al., 2007). PCD in *Trichodesmium* spp. is characterized by the loss of
49 buoyancy (collapse of gas vesicles) and increased production of TEP and aggregation
50 leading to enhanced and massive vertical flux (Bar-Zeev et al., 2013). A *Trichodesmium*
51 spp. bloom that occurred outside the VAHINE mesocosms on days 23-24 displayed
52 mechanistic features of PCD including mass mortality within 24 h, loss of gas vesicles, and
53 high production of TEP (Berman-Frank et al., 2016; Spungin et al., 2016). While we could
54 not directly quantify the export flux as no sediment traps were deployed in the lagoon
55 outside the mesocosms, the characteristics of the bloom, minimal grazer influence and the
56 demise of biomass suggests this would lead to high rates of export (Spungin et al., 2016) as
57 demonstrated in culture simulations (Bar-Zeev et al., 2013) (Fig 5c). “

58
59 TECHNICAL CORRECTIONS

60 Abstract: 9 – delete “potential”

61

62 **This word has been deleted**

63

64 p.7 11-23 – It is preferable to pose your objectives as statements rather than questions.

65 Indeed, objective iii is posed as a statement but provided with an erroneous question mark!

66 The same for the first line in objective iv.

67

68 **The objectives have been reformulated as statements:**

69 **The main scientific research priorities of the project were:**

70 **i) To quantify the DDN which enters the planktonic food web,**

71 **ii) To investigate how the development of diazotrophs influences the subsequent
72 diversity, gene expression, and production of primary producers, heterotrophic
73 bacterioplankton, and subsequently zooplankton abundance,**

74 **iii) To examine whether different functional types of diazotrophs significantly modify
75 the stocks and fluxes of the major biogenic elements (C, N, P),**

76 **iv) To elucidate whether the efficiency of particulate matter export depends on the
77 development of different functional types of diazotrophs.**

78

79 27 – consider changing “stable” for “unique”

80 p.8 14 – change “has been” to “was”. (The experiment is not ongoing; it was terminated
81 after 23 days).

82 16 change “harbouring” to “exhibiting”

83

84 **These changes have been applied.**

85

86 p.13 21 – refrain from citing your work as the “first”. If it really is, others will identify it as
87 such.

88

89 **We concur and have changed this especially on page 15.**

90

91 26 – Change “way” to “pathway”

92 p.14 1- Change “the one” to “that”

93 15- Change “The export” to “Export”; change “has not” to “was”

94 26 – Rephrase to eliminate inappropriate question mark.

95 28 – Eliminate redundancy; change second “UCYN-C” to “these”
96 p.15 6 – Rephrase to avoid “first”. Perhaps, “We thus demonstrate that UCYN blooms may
97 result in substantial DDN release.”

98
99 **The suggested changes have been applied.**

100
101

102 Referee #2

103

104 P2L11, P7L28 my experience suggests that mesocosms do disturb the ambient light
105 field. Please provide evidence to the contrary or reference to the relevant paper in the
106 special issue to support this.

107

108 **We agree that the mesocosms may have produced a slight change in the light**
109 **environment, albeit this was probably limited. So, we modified the text as follows in the**
110 **revised version of the manuscript: “...to maintain a stable water-mass minimizing the**
111 **disturbance of ambient light and temperature conditions...” . In addition, we added the**
112 **Guieu et al. (2010) reference that describe extensively the mesocosms setup used during**
113 **the VAHINE experiment and where the question has been more largely discussed.**

114

115 P3L12 tropical LNLC ecosystems include : : :subtropical gyres? Tropical and subtropical
116 are different environments/regimes

117

118 **We agree with this comment and removed “Tropical” at the beginning of the sentence.**

119

120 P3L22 need an extra) after 2008))

121 P3L29cycles HAS been

122 P3L32 preferentially exported directly

123 P5L12 phytoplankton. Actual Calculations of DDN transfer were first

124

125 **All these suggested changes have been done**

126

127 P5L21 : ...poorly and challenged qualified due mainly to

128

129 **Rephrased as follows: “remains poorly qualified and challenging”**

130

131 P9L4 ..good replicability low variability

132

133 **Rephrased as follows: “These studies also revealed a good replicability and low variability**
134 **between stocks, fluxes and plankton diversity measurements among the replicate**
135 **mesocosms. ...**

136

137 P9L18 within MESOCOSMS and in

138

139 **The word addition has been applied in the new version of the manuscript.**

140

141 P11L2 How is DIP turn-over time defined and measured. I do not see any reference to

142 DIP uptake rates. This needs some detail or referencing to the appropriate paper.

143

144 **DIP turn over time was measured using the radioisotope ^{33}P according to Duhamel et al.**
145 **(2006). Results and details on DIP turn-over time measurements during the VAHINE**
146 **experiment are presented in Berthelot et al. (2015). The references for each of the**
147 **parameters have been added in the caption of Figure 4.**

148

149 P11 A number of rate measurements are introduced on this page which do not seem
150 to be defined (e.g. APA, PP, BP) neither is there reference to the papers containing this
151 data/description.

152

153 **All acronyms are now defined in the text when they appear for the first time.**

154

155 P12L5-8 How do your results demonstrate this? No evidence is given here neither is there
156 any reference to the paper detailing this work.

157

158 **This is detailed in the Gimenez et al paper within the special issue as cited in the text.**
159 **However, we acknowledge that this part is not the main goal of the present paper and we**
160 **decided to remove this paragraph.**

161

162 P12L28-29 : : : :to determine whether : : : :of particulate matter, and if so, how was
163 this manifested.

164

165 **The suggested change has been applied.**

166

167 P12L34 equalled

168

169 **The suggested change has been applied.**

170

171 P13L27 what is nanosims and how was it used to demonstrate this?

172

173 **NanoSIMS refers to nanoscale Secondary Ion Mass Spectroscopy. The sentence has been**
174 **modified as follows: ‘An experiment performed during the UCYN bloom using nanoSIMS**
175 **(nanoscale Secondary Ion Mass Spectroscopy) as described in Bonnet et al., (2016)**
176 **demonstrated that a significant...’**

177

178 P14L2 define e-ratio

179

180 **The e-ratio depict the efficiency of the carbon export compared to primary production. In**
181 **order to clarify, we modified the text as follows: “indicated by e-ratio calculations (e-ratio**
182 **= $\text{PP}/\text{POC}_{\text{export}}$), which quantify the efficiency of a system to export particulate C relative to**
183 **the C fixed by PP).**

184

185 P14L28-32 long sentence which needs breaking up

186

187 **The sentence has been divided in two as follows: “During the maximal abundance of**
188 **UCYN-C, these were responsible for $90\pm 29\%$ of total N_2 fixation rates in the mesocosms**

189 **(Bonnet et al., 2015a). During this period, the DDN released to the dissolved pool (based**
190 **on the direct measurement of the isotopic signature (¹⁵N) of the total dissolved N**
191 **according to the denitrifying method (Knapp et al., 2005)) accounted for 7.1±1.2 to**
192 **20.6±8.1 % of gross N₂ fixation (Bonnet et al., 2015a)."**

193
194 P15L1 Are waters contained within a mesocosm natural?

195
196 **The waters present in the mesocosms were isolated from the lagoon the first day of the**
197 **experiment. The mesocosms are designed to minimize the perturbations (temperature,**
198 **light...) and reproduce as much as possible the natural environmental conditions.**
199 **Nevertheless, we agree that in the strict mean of the term, "natural" can be seen as**
200 **inappropriate and we deleted it in the new version of the manuscript.**

201
202 P15L23 Surely the evidence to date indicates that the bubble method underestimates
203 rates? (Mohr et al, Grosskopf et al, etc)

204
205 **Indeed, Mohr et al. 2010 reference appears to be more suitable in the context of the**
206 **sentence and replace the Montoya et al. 1996 reference in the new version of the**
207 **manuscript.**

208
209 P16L22-27 long sentence

210
211 **The sentence has been divided in two in the new version of the manuscript: "The**
212 **relationships between BP and N₂ fixation rates were weak (during P2) or absent (during**
213 **P1) but tightly coupled between BP and Chl *a* concentrations, and between BP and PP. This**
214 **suggests that N₂ fixation stimulated autotrophic communities and these subsequently**
215 **stimulated heterotrophic prokaryotes through the production and release of dissolved**
216 **organic matter including C (DOC) (Van Wambeke et al., 2015)."**

217
218 P17L10-15 long sentence

219
220 **The sentence has been modified as follows: "These results indicate that the extensive**
221 **oceanic blooms of *Trichodesmium* spp. can contribute to a large indirect downward flux of**
222 **organic matter by promoting large cells (e.g., diatoms and dinoflagellates) characterized by**
223 **efficient export rates (Nelson et al., 1995, Bonnet et al., Accepted; Devassy et al., 1979;**
224 **Lenes et al., 2001)."**

225
226 P19L5 deconvolute???? No idea what is meant by this

227
228 **The term "deconvolute" has been changed by "separate".**

229
230 P19L15-16 .. during the first 10 days

231 P19L31-32 (nearly up to 40% of the DDNexperiment is found)

232 P20L12 DDN was mainly transferred through

233
234 **The suggested change has been applied in the new version of the manuscript.**

235

236 Figs 6,7,8 Labelling of REF and NOFIX in figure legends is the wrong way round

237

238 **The captions of Figs. 6,7 and 8 have been corrected in order to fix this problem.**

239

240 P22L9-10 aggregation processes mediated diazotrophs-derived TEP release – this
241 needs re-phrasing somehow

242

243 **The sentence has been rephrased and the paragraph in question has been reorganized to
244 improve the clarity of the text :**

245 **“It is likely that during the experiment, TEP release favored aggregation and accumulation
246 of particles and subsequently enhanced vertical flux from the different compartments in
247 the water column. To represent the latter phenomenon, we considered in the model that
248 10 % of the living and non-living compartments were allowed to sink after day 10 (see
249 Gimenez et al. (2016) for more details). Since this extra aggregation is mainly attributable
250 to diazotrophs, it was not represented in the NOFIX simulation. However, we ran a third
251 simulation (not shown) to further analyze the excess of C export in the REF simulation as
252 compared to the NOFIX one (Fig. 8). This third simulation is intermediate between the REF
253 and the NOFIX simulations in that sense that only the N₂ fixation capability by diazotrophs
254 is removed (but aggregation processes are still represented). This simulation indicated that
255 C export is nearly equal to that of the REF simulation after 25 days (they differ by only 2.9
256 %), thereby suggesting that during the 25 first days, the suppression of N₂ fixation does
257 not significantly impact carbon export fluxes. This further suggests that the higher C export
258 in the REF simulation during P2 (Fig.8) is mainly due to aggregation processes mediated by
259 diazotrophs-derived TEP release and the subsequent export of diazotrophs (Berman-Frank
260 et al., 2016; Bonnet et al., 2015a). However, beyond day 25, the difference in carbon
261 export between the REF and the third simulation increases up to 25% on day 35. In other
262 words, the N₂ fixation process per se (by supporting PP and BP fluxes) contributes more
263 and more to the enhanced C export as N₂ fixation fluxes increase. Hence, on day 30, N₂
264 fixation supports ~50 % of the excess C export observed between the REF and the NOFIX
265 simulations, the remaining still being attributed to aggregation processes”.**

266

267 P23L26 what is PCD?

268

269 **PCD means “Programmed Cell Death” and is defined in the new version of the manuscript.**

270

271 Referee #3

272

273 The interdisciplinary VAHINE project has already generated a large number of data rich
274 papers, a dozen of which are cited in this paper. This current manuscript provides a summary
275 (synthesis) of some of the major trends from this controlled mesocosm experiment. I have
276 not gone back and read all the individual papers so I cannot really comment on the accuracy
277 or inclusive nature of this summary; hence, I do not have an informed opinion of whether it
278 is needed as a “stand alone” paper. I was surprised to learn that yet another paper (listed in
279 the reference list as Bonnet et al., in preparation) termed “Introduction to the project
280 VAHINE” is planned. It struck me as odd that no “introduction” had yet been published,
281 given the many papers that have already appeared. Why not combine the introduction and
282 the synthesis into a single paper? That would seem logical to this reader.

283
284 **Actually the Introductory paper is already published in BG discussion**
285 **(<http://www.biogeosciences-discuss.net/bg-2015-615/>) and has been recently accepted**
286 **for final publication in BG after minor revisions. We agree that it was misleading as it**
287 **appeared as ‘in prep’ in the present paper.**

288 **This intro paper aims at describing the scientific objectives of the project as well as the**
289 **implementation plan: the mesocosms description and deployment, the selection of the**
290 **study site (New Caledonian lagoon) and the logistical and sampling strategy. The main**
291 **hydrological and biogeochemical conditions of the study site before the mesocosms**
292 **deployment and during the experiment itself are also described, and a general overview of**
293 **the papers published in this special issue is presented. All papers from the special issue**
294 **could then refer to this one to avoid repeating the detailed mesocosms strategy (which**
295 **was quite complex) in their paper**

296 **The present Synthesis paper aims at summarizing the major experimental and modelling**
297 **results obtained during the project and described in the Special issue. We thus decided to**
298 **divide this in 2 distinct papers**

299

300 Specific Comments

301 p. 2, line 11: “a stable water mass” – Was turbulence measured?

302

303 **The turbulence has not been measured. We replaced the sentence by ‘The sentence has**
304 **been replaced by ‘Triplicate large volume (~ 50 m³) mesocosms were deployed in the**
305 **tropical South West Pacific coastal ocean (New Caledonia) to isolate a water-mass with**
306 **minimizing disturbance of ambient light and temperature conditions’ in the revised**
307 **version of the manuscript.....**

308

309 p. 3, line 5: ammonia is NH₃, ammonium is NH₄⁺

310

311 **“Ammonia” has been replaced by “ammonium” in the new version of the manuscript.**

312

313 p. 3, line 6: crops, not cultures?

314

315 **“Cultures” has been replaced by “crops” in the new version of the manuscript.**

316

317 p. 5, line 21: quantified, not qualified?

318

319 **“Qualified” has been replaced by “quantified” in the new version of the manuscript.**

320

321 p. 6, line 22: Eastern Tropical Pacific?

322

323 **We change to “Eastern Tropical North Pacific” as mentioned in White et al. (2012) in the**
324 **new version of the manuscript.**

325

326 p. 8, line 17: 40 nM NO₃⁻ seems high to me. So does 0.1-0.15 μA₂ g Chl a l⁻¹

327

328 **The sentence has been replaced by ‘The New Caledonian lagoon was chosen as it is a well-**
329 **studied environment (Special issue Marine Pollution Bulletin 2010 (Grenz and LeBorgne,**

330 **2010)) submitted to high oceanic influence (Ouillon et al., 2010) and exhibiting typical**
331 **LNLC conditions during the summer season (NO_3^- concentrations $<0.04 \mu\text{mol L}^{-1}$ and**
332 **chlorophyll a (Chl α) $\sim 0.10\text{-}0.15 \mu\text{g L}^{-1}$ (Fichez et al., 2010)).**

333
334 Fig. 3: Why not plot particulate P and DOP?

335
336 **We chose to present in this figure mainly the plots related to the N dynamics as this is**
337 **what is specifically discussed in the manuscript. Particulate P and DOP are both presented**
338 **in the companion paper Berthelot et al. (2015) within the special issue.**

339
340 Fig. 3: units on (h) PON export seem to be incorrect

341
342 **Indeed, the units for PON export were wrong (should be $\mu\text{mol d}^{-1}$ instead of $\mu\text{mol L}^{-1}$). The**
343 **correction has been applied to the figure and its caption in the new version of the**
344 **manuscript.**

345
346
347 References cited:

348 Duhamel, S., Zeman, F., and Moutin, T.: A dual labelling method for the simultaneous
349 measurement of dissolved inorganic carbon and phosphate uptake by marine planktonic
350 species, *Limnol. Oceanogr.-Meth.*, 4, 416–425, doi:10.4319/lom.2006.4.416, 2006.

351
352 Berthelot, H., Moutin, T., L'Helguen, S., Leblanc, K., Hélias, S., Grosso, O., Leblond, N.,
353 Charrière, B., and Bonnet, S.: Dinitrogen fixation and dissolved organic nitrogen fueled
354 primary production and particulate export during the VAHINE mesocosm experiment (New
355 Caledonia lagoon), *Biogeosciences*, 12, 4099-4112, 2015.

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366 **Biogeochemical and biological impacts of diazotroph**
367 **blooms in a Low Nutrient Low Chlorophyll ecosystem:**
368 **synthesis from the VAHINE mesocosm experiment (New**
369 **Caledonia)**

370

371 **S. Bonnet^{1,2}, M. Baklouti¹, A. Gimenez¹, H. Berthelot¹, I. Berman-Frank³**

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373

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Code de champ modifié

398 **Abstract**

399 In marine ecosystems, N₂ fixation provides the predominant external source of nitrogen (N)
400 (140±50 Tg N yr⁻¹), contributing more than atmospheric and riverine inputs to the N supply.
401 Yet the fate and magnitude of the newly-fixed N, or diazotroph-derived N (hereafter named
402 DDN) in marine ecosystems is poorly understood. Moreover, ~~it remains unclear~~ whether the
403 DDN is preferentially and directly exported out of the photic zone, recycled by the microbial
404 loop, and/or transferred into larger organisms, ~~subsequently enhancing indirect particle~~
405 ~~export, remains unclear~~. These questions were investigated in the framework of the VAHINE
406 (VAriability of vertical and tropHic transfer of diazotroph derived N in the south wEst
407 Pacific) project. Triplicate large volume (~ 50 m³) mesocosms were deployed in the tropical
408 South West Pacific coastal ocean (New Caledonia) ~~to isolate maintain a stable water mass~~
409 ~~with without disturbing minimizing disturbance of ambient light and temperature conditions~~.
410 The mesocosms were intentionally fertilized with ~0.8 μM dissolved inorganic phosphorus
411 (DIP) at the start of the experiment to stimulate diazotrophy. A total of 47 stocks, fluxes,
412 enzymatic activities and diversity parameters were measured daily inside and outside the
413 mesocosms by the 40 scientists involved in the project. The experiment lasted for 23 days and
414 was characterized by two distinct and successive diazotroph blooms: a dominance of diatom-
415 diazotroph associations (DDAs) during the first half of the experiment (days 2-14) followed
416 by a bloom of UCYN-C during the second half of the experiment (days 15-23). These
417 conditions provided a unique opportunity to compare the DDN transfer and export efficiency
418 associated with different diazotrophs. Here we summarize the major experimental and
419 modelling results obtained during the project and described in the VAHINE Special issue, in
420 particular those regarding the evolution of the main standing stocks, fluxes and biological
421 characteristics over the 23-days experiment, the contribution of N₂ fixation to export fluxes,
422 the DDN released to dissolved pool and its transfer to the planktonic food web (bacteria,
423 phytoplankton, zooplankton). We then apply our Eco3M modelling platform ~~further~~ to further
424 infer the fate of DDN in the ecosystem and role of N₂ fixation on productivity, food web
425 structure and carbon export. Recommendations for future work are finally provided in the
426 conclusion section.

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431

432 **1 Introduction**

433 Atmospheric dinitrogen (N₂) is the largest pool of nitrogen (N) on earth yet it is unavailable
434 for most organisms that require N for growth. Biological fixation of N₂ (or diazotrophy) is
435 catalyzed by the nitrogenase enzyme (encoded by the *nifH* genes) that converts the inert
436 triple-bond N₂ into bioavailable ammonium (NH₄⁺). This process has long been studied in
437 terrestrial agriculture as it increases the yield of ~~cultures~~ crops associated with N₂-fixing
438 organisms diazotrophs. In the ocean, diazotrophy provides the predominant external source of
439 N (140±50 Tg N yr⁻¹) contributing more than atmospheric and riverine inputs (Gruber, 2004).

Code de champ modifié

440 Moreover, N₂ fixation acts as a ~~potential~~ natural fertilizer adding a source of new N that is
441 available for non-diazotrophic primary producers and bacterioplankton especially in Low
442 Nutrient, Low Chlorophyll (LNLC) ecosystems, where N is the proximal limiting nutrient ~~e.g.~~
443 (Moore et al., 2013). ~~Tropical~~ LNLC ecosystems include the vast oligotrophic subtropical
444 gyres and represent more than 60 % of the global ocean area. N₂-fixing organisms ~~(or~~
445 ~~diazotrophs)~~ have a competitive advantage and sustain a large percentage (~50 %) of new
446 primary production (PP) e.g. (Karl et al., 2002) in these vast ecosystems.

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447 The non-heterocystous filamentous cyanobacterium *Trichodesmium* spp. remains the most
448 studied marine diazotroph. Based on direct rate measurements, *Trichodesmium* accounts for a
449 quarter to half of geochemically-derived estimates of marine N₂ fixation at the global scale
450 (Mahaffey et al., 2005). Diverse cyanobacteria and bacteria also fix N₂ in marine waters.

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451 These include: (1) the heterocystous cyanobacteria frequently found in association with
452 diatoms (diatom-diazotroph associations, ~~hereafter~~ referred to as DDAs; (Foster and
453 O'Mullan, 2008)) efficient at exporting organic matter out of the photic zone (Karl et al.,
454 2012), (2) unicellular cyanobacterial lineages (UCYN-A, B, and C) with a size range from 1
455 to 6 μm (Moisander et al., 2010), which are key oceanic diazotrophs (Luo et al., 2012)
456 accounting for the predominant fraction of N₂ fixation in many tropical oceans (Bonnet et al.,
457 2009; Montoya et al., 2004), and (3) non-cyanobacterial N₂-fixing bacteria and archaea that
458 are still poorly characterized yet recent studies show they are abundant and active across the
459 world's oceans (Bonnet et al., 2013; Farnelid et al., 2011; Farnelid and Riemann, 2008;
460 Moisander et al., 2014).

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461 While the role and contribution of marine N₂ fixation on biogeochemical cycles ~~has~~ ve been
462 intensely investigated, a critical question that remains poorly studied is the fate of newly-fixed
463 N, or diazotroph-derived N (hereafter named DDN) in LNLC ecosystems (Mulholland, 2007).

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464 It remains unclear whether the DDN is preferentially exported directly ~~exported~~ out of the

465 photic zone, recycled by the microbial loop, and/or transferred into larger organisms,
466 subsequently enhancing indirect particle export.

467 This question was investigated in the framework of the VAHINE (VARIability of vertical and
468 troPHic transfer of diazotroph derived N in the south wEst Pacific) project. Here we
469 summarize the major results described in the VAHINE Special issue and integrate them to
470 obtain general conclusions from the experiment. In this introduction ~~section~~, we first
471 summarize some of our knowledge regarding the fate of DDN in the ocean, describe the
472 ongoing technical challenges to study this question, and the specific scientific objectives of
473 the VAHINE project.

474

475 **1.1 Current knowledge on the fate of DDN in the ocean**

476 **1.1.1 DDN release to the dissolved pool**

477 ~~As the biologically catalysed process of N₂ fixation is not entirely efficient, d~~Diazotrophs
478 release some of the recently fixed N₂ as dissolved organic N (DON) and NH₄⁺ to the
479 surrounding waters (Glibert and Bronk, 1994; Meador et al., 2007; Mulholland et al., 2006).

480 Several studies have reported elevated DON and NH₄⁺ concentrations during and immediately
481 after *Trichodesmium* spp. blooms in the Indian (Devassy et al., 1979; Devassy et al., 1978;
482 Glibert and O'Neil, 1999), Pacific (Karl et al., 1992; Karl et al., 1997b), and Atlantic (Lenes et

483 al., 2001) oceans. Subsequent culture (Hutchins et al., 2007; Karl et al., 1992; Karl et al.,
484 1997a) and field studies (Benavides et al., 2013b; Konno et al., 2010; Mulholland and

485 Bernhardt, 2005) have quantified that diazotrophs release ~50 % of the total fixed N₂ to the
486 dissolved pool. Most of these studies were performed on the conspicuous *Trichodesmium* spp.
487 and were based on the difference between gross N₂ fixation (measured by acetylene reduction

488 assays) and net N₂ fixation (Mulholland et al., 2004) measured using the ¹⁵N₂ labelling
489 technique (Montoya et al., 1996). The recent modification of the ¹⁵N₂ labelling method (Mohr
490 et al., 2010) led to higher net N₂ fixation rates and potentially reduced the gap between gross

491 and net N₂ fixation. Applying the new N₂ fixation method and the direct measurement of the
492 ¹⁵N signature on the released DON and NH₄⁺ demonstrated low release rates from
493 *Trichodesmium* spp. and from three strains of UCYN-B and C (<1 % of total N₂ fixation)

494 (Berthelot et al., 2015a). Similar experiments (examining the direct ¹⁵N measurement on
495 released molecules) showed low release by UCYN-C (~1 %, (Benavides et al., 2013a)).
496 Culture studies probably represent lower end estimates of DDN release, as in the field,
497 exogenous factors such as viral lysis (Hewson et al., 2004; Ohki, 1999) and sloppy feeding

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498 (O'Neil et al., 1996) may enhance the leakage of DDN by UCYN, yet such field studies on
499 these organisms are rare.

500

501 **1.1.2 Transfer of DDN to the trophic chain and impact on plankton community** 502 **composition**

503 The transfer of DDN towards the first levels of the food chain (phytoplankton, bacteria) is
504 mainly achieved through the dissolved pool. Devassy et al. (1979) first observed that as
505 blooms of *Trichodesmium* spp. decayed in the Indian ocean, diatom populations increased
506 (mainly *Chaetoceros* sp.), followed by a succession of cladocerans, dinoflagellates, green

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507 algae, and finally copepods. In the Atlantic, a high abundance of non-diazotrophic diatoms
508 and dinoflagellates succeeded blooms of *Trichodesmium* spp. (Devassy et al., 1978; Furnas
509 and Mitchell, 1996; Lenés et al., 2001), while in the pelagic waters of the Kuroshio current,
510 *Trichodesmium* spp. and diatom abundance were positively correlated (Chen et al., 2011).

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511 These studies suggest a potential transfer of DDN from diazotrophic to non-diazotrophic
512 phytoplankton. Actual calculations of DDN transfer were first performed by Bronk et al.

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513 (2004), Lenés and Heil (2010) and Sipler et al. (2013), who demonstrated how the DDN
514 released by *Trichodesmium* spp. affected the bloom dynamics of the toxic dinoflagellate

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515 *Karenia brevis* in the Gulf of Mexico. Results from size-fractionation of picoplankton after
516 ¹⁵N₂ incubations also supported the idea of a DDN transfer towards non-diazotrophic plankton
517 (Bryceson and Fay, 1981; Olendieck et al., 2007; Garcia et al., 2007), (Bryceson and Fay,
518 1981; Olendieck et al., 2007; Garcia et al., 2007). Yet, this method could not discriminate
519 the DDN transfer towards non-diazotrophic picoplankton from N₂ fixation by picoplankton
520 itself and thus likely overestimated the DDN transfer.

521 Thus, the actual transfer of DDN towards non-diazotrophic phytoplankton and bacteria
522 remains poorly qualified-quantified and challenging due mainly to technical limitations as it
523 requires appropriate methodologies to track the passage of DDN through the different
524 components of microbial food web. Moreover, the planktonic groups (autotrophic versus
525 heterotrophic, small versus large phytoplankton) that benefit the most from this DDN and
526 develop during/after diazotroph blooms have not been identified so far despite their potential
527 to differentially affect-influence the structure of the trophic chain and eventually the mode of
528 carbon (C) export of-carbon (C) from the photic zone.

529 Regarding higher trophic levels, low δ¹⁵N signatures measured on zooplankton indicate that
530 DDN is transferred towards secondary producers (Montoya et al., 2002b). This transfer can be
531 direct through the ingestion of diazotrophs (O'Neil et al., 1996; Wannicke et al., 2013a), or

532 indirect, i.e. mediated by the dissolved N released by diazotrophs (Capone et al., 1994; Glibert
533 and Bronk, 1994; Mulholland et al., 2004). The dissolved N (both DIN and DON) is taken up
534 by heterotrophic and autotrophic plankton and then potentially grazed on by zooplankton, yet
535 these pathways remain poorly explored.

536 The transfer of DDN to zooplankton may possibly depend on the diazotroph community
537 composition in the water column. Toxicity of *Trichodesmium* spp. (Kerbrat et al., 2010)
538 combined with poor nutritional quality (O'Neil, 1999; O'Neil and Roman, 1992) reduce
539 grazing pressure by copepods other than ~~the several~~ harpacticoids including *Macrosetella*
540 *gracilis* (O'Neil, 1999; O'Neil and Roman, 1992). Stable isotope measurements performed on
541 zooplankton suggest higher DDN uptake when the diazotroph community is dominated by
542 DDAs rather than *Trichodesmium* spp. (Montoya et al., 2002a). Grazing experiments on
543 UCYN have not been conducted so far and the potential of UCYN as a conduit of DDN into
544 marine food webs remains unexplored.

545

546 1.1.3 Export of DDN out of the photic zone

547 Low $\delta^{15}\text{N}$ signatures in particles from sediment traps in the tropical North Pacific suggests
548 that at least part of the DDN is ultimately exported out of the photic zone (Karl et al., 2012;
549 Karl et al., 1997b; Scharek et al., 1999a; Sharek et al., 1999b). The export of DDN may either
550 be direct through sinking of diazotrophs, or indirect, through the transfer of DDN to non-
551 diazotrophic plankton in the photic zone, that is subsequently exported. While ~~it has been~~
552 ~~demonstrated that~~ DDAs can directly contribute to particle export (Karl et al., 2012;
553 Subramaniam et al., 2008; Yeung et al., 2012), the DDN export efficiency appears to depend
554 on the diazotroph community composition present in surface waters.

555 The positive buoyancy of *Trichodesmium* spp. probably prevents its downward flux and
556 settling in sediment traps (Capone et al., 1997; Walsby, 1992), although programmed cell
557 death (PCD) causing bloom demise can cause rapid export of *Trichodesmium* biomass to
558 depth (Bar-Zeev et al., 2013; Berman-Frank et al., 2004; Spungin et al., In review, 2016). In
559 the Eastern Tropical North Pacific~~north-east Pacific~~, when the diazotrophic community was
560 dominated by UCYN-A and *Trichodesmium* spp., N_2 fixation contributed ~10 % of the export
561 (White et al., 2012); when DDAs dominated the diazotrophic community they contributed
562 ~44 % of export production, thereby suggesting that DDAs have a higher export efficiency
563 compared to *Trichodesmium* spp. and UCYN-A. Despite their recent recognition as key
564 oceanic diazotrophs (Luo et al., 2012), the export efficiency of UCYN from other lineages

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565 (UCYN-B and UCYN-C) is currently undetermined as no published studies of natural
566 UCYN-B and C blooms and their fate in the ocean are [available](#) to date ~~available~~.

567 The determination of direct *versus* indirect export requires diazotroph quantification in both
568 the water column and in sediment traps in addition to clarifying the actual transfer of DDN to
569 the different groups of autotrophic and heterotrophic plankton. Few studies have thus focused
570 on the direct coupling between N₂ fixation and particulate export in general (see references
571 above). Ideally such studies require the successful encounter of an oceanic diazotroph bloom,
572 deployment of sediment traps, and long-term (several weeks) monitoring of the
573 biogeochemical characteristics of the water body influenced by the bloom, which are rarely
574 accomplished. The patchy distribution of diazotrophs in the surface ocean (Bombar et al.,
575 2015), the temporal lag between production and export, and [the](#) hydrodynamic features that
576 may decouple production in surface and export below the photic zone (Buesseler et al., 2007)
577 also make these studies very challenging.

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579 1.2 Scientific objectives of the VAHINE project

580 The main scientific research priorities of the project were:

- 581 v) To quantify the DDN which enters the planktonic food web,
- 582 vi) To investigate how the development of diazotrophs influences the subsequent
583 diversity, gene expression, and production of primary producers, heterotrophic
584 bacterioplankton, and subsequently zooplankton abundance,
- 585 vii) To examine whether different functional types of diazotrophs significantly modify the
586 stocks and fluxes of the major biogenic elements (C, N, P),
- 587 viii) To elucidate whether the efficiency of particulate matter export depends on the
588 development of different functional types of diazotrophs.

589 ~~Thus, the main scientific objectives of the VAHINE project were:~~

- 591 ~~i) To quantify the DDN which enters the planktonic food web; Is whether it is DDN~~
592 ~~preferably transferred to large size (e.g. diatoms), small size (pico-, nanophytoplankton)~~
593 ~~phytoplankton, or to the microbial food web? . What To quantify which percentage of DDN is~~
594 ~~transferred to zooplankton? . To determine Does whether the fate of the DDN it depends on~~
595 ~~the diazotroph community composition.?~~

597 ~~ii) To investigate how the development of diazotrophs influences the subsequent diversity,~~
598 ~~gene expression, and production of primary producers, heterotrophic bacterioplankton, and~~
599 ~~subsequently the zooplankton abundance.~~
600 ~~iii) To examine whether different functional types of diazotrophs significantly modify the~~
601 ~~stocks and fluxes of the major biogenic elements (C, N, P)?~~
602 ~~iv) To elucidate whether the efficiency of particulate matter export depends on the~~
603 ~~development of different functional types of diazotrophs and whether the? Is this export is~~
604 ~~direct (through the sinking of diazotrophic cells) or indirect (through the transfer of DDN to~~
605 ~~non-diazotrophic plankton that is subsequently exported)?~~
606

607 To achieve these goals and concurrently determine N₂ fixation and particle export, we isolated
608 large water masses containing ambient planktonic communities by deploying three large-
609 volume (~50 m³) mesocosms (Bonnet et al., 2016b) thereby maintaining a ~~stable-unique~~
610 water-mass ~~with minimal disturbance of the *in-situ* light and without minimizing~~
611 ~~disturbance of ambient light and~~ temperature conditions (Guieu et al., 2016). The
612 experimental location in the southwestern Pacific region was chosen as in this area some of
613 the highest rates of oceanic N₂ fixation occur (Bonnet et al., 2015; Messer et al., 2015).
614 Additionally, to enhance N₂ fixation, the mesocosms were intentionally fertilized with
615 dissolved inorganic phosphorus (DIP). The experiment lasted 23 days and was characterized
616 by a dominance of DDAs during the first half of the experiment (days 2-14) and a bloom of
617 UCYN-C during the second half of the experiment (days 15-23), providing a unique
618 opportunity to compare the DDN transfer and export efficiency associated with specific
619 diazotrophs in this experimental system. Some additional process experiments performed on
620 *Trichodesmium* spp. which bloomed outside the mesocosms on the last two days are also
621 presented here.

622 Below, we summarize the scientific strategy used in this study, as well as some of the major
623 results obtained during this project and propose some scientific perspectives for the future.

624

625 **2 Scientific strategy**

626 **2.1 Brief description of the mesocosms and study site**

627 The large-volume (~50 m³) mesocosm experiment was undertaken in New Caledonia, located
628 1500 km east of Australia in the Coral Sea (southwestern tropical Pacific, Fig. 1). Three
629 replicate polyethylene and vinyl acetate mesocosms (diameter 2.3 m, height 15 m, volume
630 ~50 m³, Fig. 2) were deployed 28 km off the coast of New Caledonia at the entrance to the

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631 Noumea coral lagoon (22°29.073 S - 166°26.905 E) for 23 days from January 13th to February
632 6th (austral summer). The New Caledonian lagoon ~~has been was~~ chosen as it is a well-studied
633 environment (Special issue Marine Pollution Bulletin 2010 (Grenz and LeBorgne, 2010))
634 submitted to high oceanic influence (Ouillon et al., 2010) and ~~harbouring exhibiting~~ typical
635 ~~oligotrophic-LNLC~~ conditions during the summer season (NO_3^- concentrations $<0.04 \mu\text{mol L}^{-1}$
636 and chlorophyll a (Chl *a*) $\sim 0.10\text{-}0.15 \mu\text{g L}^{-1}$ (Fichez et al., 2010). Primary productivity is N-
637 limited throughout the year (Torréton et al., 2010), giving diazotrophs a competitive
638 advantage. New Caledonian waters support high N_2 fixation rates ($151\text{-}703 \mu\text{mol N m}^{-2} \text{d}^{-1}$,
639 (Garcia et al., 2007)), as well as high *Trichodesmium* spp. (Dupouy et al., 2000; Rodier and
640 Le Borgne, 2010, 2008), and UCYN abundances (Biegala and Raimbault, 2008), therefore
641 representing an ideal location to implement the VAHINE project and study the fate of DDN in
642 the marine ecosystem.

643 DIP availability can control N_2 fixation in the southwestern Pacific (Moutin et al., 2008;
644 Moutin et al., 2005), hence the mesocosms were intentionally fertilized with $\sim 0.8 \mu\text{M}$ DIP
645 (KH_2PO_4) ~~on t~~the evening of day 4 to alleviate any potential DIP limitation and promote N_2
646 fixation and even diazotroph blooms for the purpose of the project.

647 The mesocosms used for this study are well suited for conducting replicated process studies
648 on the first levels of the pelagic food web (Bonnet et al., 2016b; Guieu et al., 2010; Guieu et
649 al., 2014). They are equipped with sediment traps allowing the collection of sinking material.
650 Due to the height of the mesocosms (15 m), they do not represent processes occurring in the
651 full photic layer but allow studying the dynamics of C, N, P pools/fluxes and export
652 associated with the plankton diversity in the same water mass, and comparing these dynamics.
653 before/after the DIP fertilization, and under contrasted conditions regarding the diazotroph
654 community composition (cf below). Detailed surveys performed in LNLC environments
655 revealed that temperature and light conditions are not affected by the presence of the
656 mesocosms compared to surrounding waters (Bonnet et al., 2016b; Guieu et al., 2010; Guieu
657 et al., 2014). These studies also revealed a good replicability ~~and low variability of between~~
658 stocks, fluxes and plankton diversity measurements among the replicate mesocosms. Hence,
659 the discussion below will consider the average between the three mesocosms deployed in this
660 study.

661

662 2.2 Sampling strategy and logistics

663 A complete description of the mesocosms design and deployment strategy is given in the
664 introductory article (Bonnet et al., 2016b). In total, over 47 stocks, fluxes, enzymatic activities

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665 and diversity parameters were measured daily by the 40 scientists involved in the project.
666 Protocols for each measured parameter are detailed in the specific contributions to this special
667 issue and will not be described here. Modelling has also accompanied all steps of the project
668 (see Gimenez et al. (In review, 2016) and section 5 below).

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669 Sampling for stocks, fluxes and plankton diversity measurements was performed daily at 7 am
670 in each of the three mesocosms (M1, M2 and M3) and in surrounding waters (hereafter called
671 ‘lagoon waters’) from day 2 (January 15th, the day of the mesocosms closure) to day 23
672 (February 6th) at three selected depths (1, 6 and 12 m) to study the vertical variability within
673 mesocosms and in lagoon waters. For flux measurements, bottles were incubated on an in situ
674 mooring line at the appropriate sampling depth set up close to the mesocosms. Vertical CTD
675 profiles were then performed daily at 10 am in every mesocosm and in lagoon waters using a
676 SBE 19 plus Seabird CTD to obtain the vertical profiles of temperature, salinity and
677 fluorescence. Finally, sediment traps were collected daily by SCUBA divers at 10:30 am, see
678 details in Bonnet et al. (2016b).

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679

680 **3 Evolution of the main standing stocks, fluxes and biological** 681 **characteristics during the VAHINE experiment**

682 Initial hydrological and biogeochemical conditions (i.e. conditions in ambient waters the day
683 of mesocosms deployment - January 13th, day 0) were typical of those encountered in the
684 oligotrophic Noumea lagoon during austral summer conditions (Fichez et al., 2010; Le
685 Borgne et al., 2010), with seawater temperature of 25.5°C, surface salinity of 35.15, NO₃⁻-
686 depleted waters (0.04±0.01 μmol L⁻¹), low DIP concentrations (0.04±0.01 μmol L⁻¹), and Chl
687 *a* concentrations of 0.20 μg L⁻¹. N₂ fixation rates were 8.70±1.70 nmol N L⁻¹ d⁻¹ and the
688 diazotroph community was dominated by DDAs (het-1 3.1 x 10⁴ *nifH* copies L⁻¹ and het-2 1.2
689 x10⁴ *nifH* copies L⁻¹) as well as UCYN-A2 (1.5 x 10⁴ *nifH* copies L⁻¹) and UCYN-A1 (5.6 x
690 10³ *nifH* copies L⁻¹), which together accounted for 95 % of the total *nifH* pool in the lagoon
691 waters prior to the mesocosms closure (Turk-Kubo et al., 2015).

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692 During the 23-days VAHINE mesocosm experiment, three major periods could be defined
693 based on the main C, N, P stocks and fluxes (Berthelot et al., 2015b) and on the identity of the
694 most abundant diazotrophs that developed in the mesocosms (Turk-Kubo et al., 2015): **P0**
695 from days 2 to 4 (i.e. prior to the DIP fertilization that occurred on the evening of day 4), **P1**
696 from days 5 to 14, and **P2** from days 15 to 23 (Figs. 3 and 4). Figure 3 reports the main
697 hydrological and biogeochemical parameters during the experiment. Figure 4 provides a

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698 synoptic view of the main changes (positive, negative, neutral) in the major stocks, fluxes,
699 and plankton community composition measured during P1 and P2 respectively.

700 Seawater temperature (Fig. 3) gradually increased both inside and outside the mesocosms
701 over the 23-days of the experiment from 25.5°C to 26.2°C on day 23, which is the general
702 trend observed during austral summer conditions (Le Borgne et al., 2010). The water column
703 was well homogenized inside the mesocosms throughout the experiment (Bonnet et al.,
704 2016b). NO_3^- concentrations remained close to detection limit of conventional micromolar
705 methods ($0.02 \mu\text{mol L}^{-1}$) both inside and outside the mesocosms throughout the 23 days of the
706 experiment (Fig. 3). The low ($0.04 \mu\text{mol L}^{-1}$) DIP concentrations measured during P0
707 increased in the mesocosms right after the fertilization up to $\sim 0.8 \mu\text{mol L}^{-1}$, then decreased
708 quickly to reach values close to initial DIP concentrations ($\sim 0.04 \mu\text{mol L}^{-1}$) at the end of the
709 experiment.

710 ~~As a~~ major objective of the experiment was to study the development of diazotroph blooms
711 and the fate of DDN. ~~Thus, our~~ investigation of the biological response ~~was~~ focused on
712 diazotrophs and their subsequent influence on biological and biogeochemical signatures. N_2
713 fixation rates tripled between P1 and P2, to reach extremely high rates during P2 (27.3 ± 1.0
714 $\text{nmol N L}^{-1} \text{d}^{-1}$ on average and up to $70 \text{ nmol N L}^{-1} \text{d}^{-1}$ (Bonnet et al., 2016a)) (Fig. 3), ranking
715 among the highest rates reported in marine waters (Luo et al., 2012). ~~The DDAs dominated~~
716 ~~the~~ diazotroph community composition ~~was dominated by DDAs~~ during P1, and a bloom of
717 UCYN-C occurred during P2 (Fig. 4). Standing stocks of Chl *a* and particulate organic N
718 (PON) increased by a factor of 3 and 1.5 between P1 and P2 and subsequently, export of PON
719 dramatically increased (by a factor of 5) in the mesocosms during P2 (Fig. 3). These results
720 emphasize that the experimental mesocosm setup provided ideal conditions to study the fate
721 of DDN associated with different diazotroph communities (DDAs *versus* UCYN-C).

722 The synoptic view of the mesocosm dynamics (Fig. 4) indicates that after the DIP
723 fertilization, DIP concentrations and DIP turn-over time increased significantly during P1, and
724 alleviated P-limitation in the microbial communities as reflected in the significant decline in
725 alkaline phosphatase activity (APA). The major biomass-indicative standing stock parameters
726 (Chl *a*, POCPON, PON, POP particulate organic C (POC) and P (POEP)) did not increase
727 immediately after the DIP fertilization (P1) but during P2 (see below). Only PP increased
728 significantly by a factor of 2 during P1, associated with a significant increase in N_2 -fixing
729 DDAs and *Prochlorococcus* abundances. During P1, enhanced DIP availability enabled non-
730 diazotrophic organisms with lower energetic requirements and higher growth rates such as
731 *Prochlorococcus* to outcompete the diazotrophs in the mesocosms via utilization of recycled

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732 N derived from N₂ fixation (Bonnet et al., 2016a). Thus, while PP increased, N₂ fixation rates
733 decreased significantly after the DIP spike.

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734 During P2, diazotrophy was characterized by the significant increase in UCYN-C abundances
735 that reached up to 7×10^5 *nifH* copies L⁻¹, concomitant with the utilization of DIP and the
736 significant decline in DIP concentrations, DIP turn-over time, and a parallel increase of total
737 APA. In all three mesocosms, the increase in UCYN-C abundances coincided with the day at
738 which the DIP turnover time declined below 1 d, indicative of DIP limitation (Berthelot et al.,
739 2015b; Moutin et al., 2005). UCYN-C may have also utilized dissolved organic phosphorus
740 (DOP) as a P source (Bandyopadhyay, 2011), driving the significant decline in DOP
741 concentrations observed during P2 (Berthelot et al., 2015b), Fig. 4). The mesocosm approach
742 also enabled the calculation of *in situ* growth rates for UCYN-C. These reached which were
743 up to ~ 2 d⁻¹ during P2, i.e. higher than growth rates of any other diazotrophic phylotypes
744 during P2 (Turk-Kubo et al., 2015), and indicating that, under NO₃⁻ depletion and low DIP
745 availability, UCYN-C was the most competitive diazotroph in the mesocosms.

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746
747 Under the high N₂ fixation conditions encountered during P2 (27.3 ± 1.0 nmol N L⁻¹ d⁻¹), all
748 standing stocks (Chl *a*, POC, PON, POP) increased in the mesocosms, together with PP and
749 BP (Fig. 4). The corresponding NO₃⁻, DIP, DON and DOP stocks for P2 decreased, indicating
750 active consumption by the planktonic communities. As no external supply of NO₃⁻ was
751 provided to the enclosed mesocosms, we calculated that the consumption of the NO₃⁻ stock
752 initially present in the mesocosms ($0.04 \mu\text{mol L}^{-1}$) represented less than 11 % of the integrated
753 N₂ fixation rates. Therefore, N₂ fixation supplied nearly all of the new production during the
754 experiment. Our results demonstrate that in oligotrophic N-depleted systems, as long as DIP
755 does not limit N₂ fixation (Berthelot et al., 2015b), diazotrophs can provide enough new N to
756 sustain high PP rates (exceeding $2 \mu\text{mol C L}^{-1} \text{d}^{-1}$) and high biomass ($\sim 10 \mu\text{mol L}^{-1}$ of POC
757 and $0.7 \mu\text{g L}^{-1}$ of Chl *a*), as long as DIP does not limit N₂ fixation (Berthelot et al., 2015b).
758 Furthermore, during P2, DON provided an additional N source for non-diazotrophic
759 phytoplankton and bacteria (Berthelot et al., 2015).

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760 ~~The time lag between the DIP fertilization and the increase in biogeochemical stocks/fluxes~~
761 ~~was 10 days, indicating that 10 days were necessary for N₂ fixation to sustain the high~~
762 ~~production rates observed, and to see an effective accumulation of biomass. Our results~~
763 ~~demonstrate the restricted applicability of nutrient addition experiments in small volume~~
764 ~~microcosms (several liters) mostly limited to 24-72 h incubations that are typically employed~~
765 ~~to assess nutrient limitations on plankton growth in the ocean, e.g. (Moore et al., 2013). If~~

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766 ~~indeed a longer time scale (weeks) is required to study nutrient limitation of plankton in~~
767 ~~marine ecosystems, then large volume mesocosms, such as we demonstrate here, would be~~
768 ~~more suitable (Gimenez et al., 2016).~~

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769 Concurrent with the development of diazotrophic (UCYN-C) populations, the abundance of
770 *Synechococcus*, pico-eukaryote, and nano-eukaryote primary producers also increased at the
771 end of P2 (i.e. around day 16) (Leblanc et al., In review, 2016). The non-diazotrophic diatoms
772 responded rapidly (i.e. around day 10-11) and increased to bloom values ($100,000 \text{ cells L}^{-1}$)
773 simultaneously with the UCYN-C bloom on days 15-16 and prior to the increases in the pico-
774 and nanophytoplankton (Pfreundt et al., 2016; Van Wambeke et al., Accepted). A drastic
775 change in the diatom community structure paralleled the UCYN-C bloom with an almost
776 monospecific bloom dominated by *Cylindrotheca closterium*. ~~This increase was paralleled by a~~
777 ~~drastic change in the diatom community structure, which became almost monospecifically~~
778 ~~dominated by *Cylindrotheca closterium*.~~ Despite the significant increase in BP during P2 and
779 enrichments in the 16S transcripts of specific bacterial groups (Pfreundt et al., In review,
780 2016), the total abundance of heterotrophic bacteria did not change (Van Wambeke et al.,
781 Accepted), probably due to grazing. Finally, no consistent temporal pattern in zooplankton
782 biomass was detected over the course of the experiment (Hunt et al., Accepted), although
783 changes were observed regarding the contribution of DDN to zooplankton biomass (see
784 below).

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786 4. Tracking the fate of N₂ fixation

787 4.1. Contribution of N₂ fixation to export fluxes

788 We specifically utilized the mesocosm approach to ~~answer~~ determine whether the
789 composition of the diazotroph community influenced the subsequent export of particulate
790 matter, and if so, how ~~was this was manifested~~. During P1, DDAs dominated the diazotroph
791 community. For this time period, the biomass indices (Chl *a*, POC, PON, POP) were stable
792 within the mesocosms (Fig. 3, 4), suggesting that the DDN associated with DDAs remained
793 within the symbiotic associations (i.e. was poorly transferred to the rest of the planktonic
794 community). Moreover, the amount of recently fixed N₂ ~~equalled~~ equaled that of exported
795 PON, suggesting that the recently fixed N₂ by DDAs was rapidly exported (Fig. 5a) as also
796 was also observed for DDAs in the tropical North Pacific at Station ALOHA (Karl et al.,
797 2012). DDAs such as het-1 (*Richelia* in association with the diatom *Rhizosolenia* spp.), which
798 dominated the DDA community during P1 in the mesocosms (Turk-Kubo et al., 2015) have
799 indeed been shown to sink at high rates in the ocean (Scharek et al., 1999a).

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800 During P2 and the UCYN-C bloom, the increases in Chl *a*, POC, PON, and POP
801 concentrations in the mesocosms suggest that a fraction of the recently produced biomass
802 sustained by N₂ fixation remained in the water column. The mesocosms enabled us to
803 determine whether export associated with diazotrophs was direct (through the sinking of
804 diazotrophic cells) or indirect (through the transfer of DDN to non-diazotrophic plankton that
805 is subsequently exported). The direct export of UCYN has rarely been studied (White et al.,
806 2012). Yet, UCYN contribution to vertical flux and export was assumed to be lower than the
807 contribution of DDAs due to their small size of (1 to 6 μm) and low sinking rates compared to
808 DDAs (up to 500 μm comprised of dense silica shells). qPCR quantification of diazotrophs in
809 the sediment traps revealed that ~10 % of UCYN-C from the water column was exported to
810 the traps daily, representing as much as 22.4±5.5 % of the total POC exported at the height of
811 the UCYN-C bloom (Bonnet et al., 2016a). Mechanistically, the vertical downward flux was
812 enabled by the aggregation of the small (5.7±0.8 μm) UCYN-C cells into large (100-500 μm)
813 aggregates, the size of which increased with depth (Fig. 5b) possibly due to a sticky matrix
814 composed also of transparent exopolymeric particles (TEP), ~~TEP~~ TEP which concentrations
815 increased during P2 (Fig. 4) providing both a nutrient source and aggregation enhancing
816 substrate (Berman-Frank et al., 2016). These data, reported for the first time from the
817 VAHINE experiment (Bonnet et al., 2016a), emphasize that despite their small size relative to
818 DDAs, UCYN-C are able to directly export organic matter to depth, indicating that these
819 small organisms should be considered in future biogeochemical studies.

820 The direct export of UCYN-C and other diazotrophs could not solely explain the very high
821 exported matter observed during P2 (Bonnet et al., 2016a), suggesting another pathway of
822 export during that period. An experiment performed during the UCYN bloom using
823 nanoSIMS (nanoscale Secondary Ion Mass Spectroscopy) as described in Bonnet et al.,
824 (2016) demonstrated that a significant fraction of DDN (21±4 %) was quickly (within 24 h)
825 transferred to non-diazotrophic plankton (~~Bonnet et al., 2015a~~), revealing that N₂ fixation
826 was fuelling non-diazotrophic plankton growth in the water column (Fig. 5b), suggesting an
827 indirect export pathway in addition to the direct export of UCYN-C. The fact that UCYN-C
828 fuelled non-diazotrophic plankton during P2 is consistent with the increase in biomass
829 indicators as well as the increase in non-diazotrophic phytoplankton abundances (diatom and
830 picoplankton) simultaneously with or after the UCYN-C bloom during P2.

831 The high export efficiency associated with the UCYN-C bloom compared to ~~the one that~~
832 associated with the DDAs during VAHINE was also indicated by *e*-ratio calculations (*e*-ratio
833 = POC_{export}/PP), which quantify the efficiency of a system to export POC-particulate C

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834 relative to ~~PP~~the C fixed by PP. During P2, the *e*-ratio was significantly ($p < 0.05$) higher (i.e.,
835 during the UCYN-C bloom; 39.7 ± 24.9 %) than during P1 (i.e., when DDAs dominated the
836 diazotrophic community; 23.9 ± 20.2 %) (Berthelot et al., 2015b). $\delta^{15}\text{N}$ measurements on
837 DON, PON and particles from sediment traps further substantiated these results with a
838 significantly ($p < 0.05$) higher contribution of N_2 fixation to export production during P2
839 (56 ± 24 % and up to 80 % at the end of the experiment) compared to P1 (47 ± 6 %) (Knapp et
840 al., 2015). The contribution of N_2 fixation to export (up to 80 %) was very high in our study
841 compared with reports from other tropical and subtropical regions where active N_2 fixation
842 contribute 10 to 25 % to export production (e.g. (Altabet, 1988; Knapp et al., 2005)). This is
843 consistent with the extremely high N_2 fixation rates measured in the mesocosms (up to 70
844 $\text{nmol N L}^{-1} \text{d}^{-1}$) and compared to those measured ~~in~~from other regions (Luo et al., 2012).
845 ~~The e~~Export associated with *Trichodesmium* spp. ~~has not been~~was not studied in the present
846 mesocosm experiment as only limited numbers of *Trichodesmium* spp. were counted in the
847 mesocosms (Turk-Kubo et al. 2015). Its potential for export is discussed below based on
848 parallel studies from the region and intensive short-term experiments on surface blooms of
849 *Trichodesmium* that appeared outside the mesocosms on days 22-23 (Spungin et al., In
850 review, 2016).

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852 4.2. DDN release and transfer to the food web

853 4.2.1 DDN release and transfer to non-diazotrophic phytoplankton and bacteria

854 ~~As part of VAHINE, we~~Within VAHINE we also assessed the quantity of DDN entering the
855 planktonic food web as a function of the dominant diazotroph players, and examined which
856 planktonic communities benefited the most from the DDN (i.e. small *versus* large
857 phytoplankton, or microbial food web?).

858 Diazotrophs transfer DDN to phytoplankton and heterotrophic prokaryotes via the dissolved
859 N pool (DON and NH_4^+). During the maximal abundance of UCYN-C, ~~UCYN-C~~these were
860 responsible for 90 ± 29 % of total N_2 fixation rates in the mesocosms (Bonnet et al., 2016a).
861 ~~and~~During this period, the DDN released to the dissolved pool accounted for 7.1±1.2 to
862 20.6±8.1 % of gross N_2 fixation (Bonnet et al., 2016a) (based on the direct measurement of
863 the isotopic signature (^{15}N) of the total dissolved N according to the denitrifying method
864 (Knapp et al., 2005)) ~~accounted for 7.1±1.2 to 20.6±8.1 % of gross N_2 fixation (Bonnet et al.,~~
865 2015a). This proportion is higher than that reported for UCYN-C in monospecific cultures
866 using an equivalent method (1.0 ± 0.3 to 1.3 ± 0.2 % of gross N_2 fixation (Benavides et al.,
867 2013a; Berthelot et al., 2015a). ~~In the natural waters of the mesocosms,~~ At the same time as

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868 UCYN-C bloomed, the diverse diazotroph community present in the mesocosms (Turk-Kubo
869 et al., 2015) also contributed to the DDN release. ~~was found at the same time as UCYN-C~~
870 (Turk-Kubo et al., 2015). Additionally, exogenous factors such as viral lysis (Fuhrman, 1999)
871 and sloppy feeding (O'Neil and Roman, 1992) occur in natural populations and could enhance
872 N release compared to the mono-culture studies. Here, we~~We thus demonstrate that natural~~
873 UCYN blooms may result in substantial DDN release to the marine environment.~~To our~~
874 knowledge, these data are the first reported of DDN released in a UCYN bloom.

875 The physiological state of cells probably plays a critical role in the quantity and availability of
876 DDN to the microbial communities as demonstrated in a study (applying identical
877 methodology) from two naturally-occurring blooms of *Trichodesmium* spp. in the same area
878 (New Caledonian lagoon) (Bonnet et al., Accepted). DDN release from these blooms was
879 slightly higher (bloom 1: 20±5 to 48±5 % and bloom 2: 13±2 to 28±6 % of gross N₂ fixation)
880 compared to UCYN-C (Bonnet et al., Accepted). A decaying *Trichodesmium* spp. bloom
881 (Bloom 1) was decaying, leading lead to high DDN release rates and high NH₄⁺ accumulation
882 (up to 3.4 μM) in the dissolved pool, ~~while we did not observe this in exponentially growing~~
883 *Trichodesmium* (Bloom 2), which was not observed during bloom 2 when *Trichodesmium*
884 spp. were in exponential growing phase. The importance of physiological status rather than
885 specific diazotroph types was further substantiated in earlier *Trichodesmium* culture study
886 studies (Mulholland et al., 2004; Mulholland and Capone, 2000) and showing no significant
887 differences in similar DDN release between *Trichodesmium* spp. and three strains of UCYN-B
888 and C were found by Berthelot et al. (2015a).

889 Previous comparisons between gross and net N₂ fixation rates indicated high DDN release
890 rates for oceanic populations of *Trichodesmium* spp. (40-50 % of gross N₂ fixation on
891 average, and up to 97 %, (Mulholland, 2007) and references therein). The physiological status
892 of these populations may have influenced the fluxes. Furthermore, the values could reflect a
893 methodological overestimation due to the use of the ¹⁵N₂ bubble method (Großkopf et al.,
894 2012; Montoya et al., 1996) that may lead to greater differences between gross and net N₂
895 fixation (see introduction). Currently, direct measurement of the ¹⁵N signature of the
896 dissolved N pool itself (either the TDN pool through the Knapp et al. (2005) method or both
897 the NH₄⁺ and the DON using the Slawyk and Raimbault (1995) method) appears the preferred
898 method to accurately quantify the amount of DDN released by diazotrophs in the dissolved
899 pool (Berthelot et al., 2015a).

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901 Once released in the form of NH_4^+ and/or DON, DDN can be taken up by surrounding
902 planktonic communities. Experimental evidence from nanoSIMS experiments during
903 VAHINE indicate that 21 ± 4 % of the $^{15}\text{N}_2$ fixed during the UCYN-C bloom was transferred
904 to the non-diazotrophic plankton after 24 h of incubation (Bonnet et al., 2016a). Among these
905 21 ± 4 %, 18 ± 3 % was transferred to picoplankton (including both pico-phytoplankton and
906 heterotrophic prokaryotes) and 3 % to diatoms (Fig. 5b), suggesting that picoplankton would
907 be more competitive than diatoms using DDN, which is consistent with the increase in
908 *Synechococcus* and pico-eukaryote abundances by a factor of two following the UCYN-C
909 bloom (Leblanc et al., In review, 2016; Pfreundt et al., 2016). The short-term nanoSIMS
910 experiment was performed on day 17, when pico- and nanoplankton dominated the
911 phytoplanktonic biomass and diatom abundances declined probably due to DIP limitation
912 (Leblanc et al., In review, 2016). Picoplankton can efficiently utilize low DIP concentrations
913 (Moutin et al., 2002) and/or can use alternative DOP sources (Benitez-Nelson and Buesseler,
914 1999) (~~Pfreundt et al., 2016; Van Wambeke et al., 2015~~). ~~This, which~~ may explain why ~~they~~
915 picoplankton were the first beneficiaries of the DDN from UCYN-C ~~at that time of~~
916 ~~the specifically from days 17-23 mesocosm experiment~~, although we cannot exclude that
917 diatoms had also benefited from the DDN from UCYN-C ~~but~~ earlier in the experiment
918 (between days 10-11 and days 15-16 when they reached bloom values of $\sim 100\,000$ cells L^{-1}),
919 ~~when the DIP turn over time was still higher than 1 d (indicative of no DIP limitation,~~
920 ~~(Berthelot et al., 2015b))~~.

921 A significant increase of both PP and BP during P2 (Fig. 2) suggests that both autotrophic and
922 heterotrophic communities benefited from the DDN (Bonnet et al., 2016a). Calculations based
923 on C:N molar ratios show that N_2 fixation may have provided ~ 30 % of the N demand of the
924 N-limited bacteria during P2 (compared to ~ 20 % during P1), the rest ~~being likely~~ provided
925 by detritus and DON (Van Wambeke et al., Accepted), which concentrations decreased during
926 the 23 days (Berthelot et al., 2015b). ~~Throughout VAHINE, the~~ The biological system inside
927 the mesocosms was net autotrophic ~~during VAHINE~~, with an upper error limit close to the
928 metabolic balance between autotrophy and heterotrophy (Van Wambeke et al., Accepted).

929 The ~~weak (during P2) or absent (during P1) correlations-relationships~~ between BP and N_2
930 fixation rates ~~were weak (during P2) or absent (during P1) and the~~ but yet tightly coupled
931 ~~relationships~~ between BP and Chl *a* concentrations, and between BP and PP. ~~This~~ suggests
932 that N_2 fixation stimulated autotrophic communities and these subsequently ~~stimulated-fueled~~
933 heterotrophic prokaryotes through the production and release of dissolved organic matter
934 including C (DOC) (Van Wambeke et al., Accepted).

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935 In a recent study performed at the VAHINE study site, (Berthelot et al., In review, 2016)
936 compared the DDN transfer efficiency to several groups of non-diazotrophic plankton as a
937 function of the diazotroph groups dominating the community (*Trichodesmium* spp. versus
938 UCYN-B versus UCYN-C). Simulated blooms of *Trichodesmium* spp., UCYN-B and UCYN-
939 C grown in culture added to ambient lagoon communities reveal that the primary route of
940 transfer of DDN towards non-diazotrophs is NH_4^+ , and DON mainly accumulates in the
941 dissolved pool, whatever the diazotroph considered. In all cases, the presence of diazotrophs
942 stimulated biomass production of non-diazotrophs, with heterotrophic prokaryotes the main
943 DDN beneficiaries of the DDN followed by diatoms and picophytoplankton. NanoSIMS
944 analyses revealed that heterotrophic prokaryotes were highly ^{15}N -enriched, confirming they
945 can directly benefit from the DDN (Berthelot et al., In review, 2016). Further studies are
946 needed to study the indirect stimulation of heterotrophic prokaryotes through the release of
947 DOC by diazotrophs and non-diazotrophic phytoplankton that that has been were -stimulated
948 by the DDN.

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949 Similar experiments ($^{15}\text{N}_2$ labelling, flow cytometry cell sorting and nanoSIMS) performed on
950 three naturally-occurring *Trichodesmium* spp. blooms in the southwestern Pacific illustrated
951 that DDN was predominantly transferred to diatoms ~~whose abundance increased from 1.5 to~~
952 ~~15 fold during and after the *Trichodesmium* spp. blooms~~ (Bonnet et al., Accepted). These
953 results e results from these small scale experiments indicate that under realistic conditions the
954 extensive oceanic blooms of *Trichodesmium* spp. ~~(reaching tens to thousands of km^2), the~~
955 ~~high amounts of DDN can fuel successively large diatom or dinoflagellate blooms, whose~~
956 ~~efficient export rates (Nelson et al., 1995)~~ can contribute to a large indirect downward flux of
957 organic matter by promoting large cells (e.g., diatoms and dinoflagellates) characterized by
958 efficient export rates (Nelson et al., 1995, Bonnet et al., Accepted; Devassy et al., 1979; Lenex
959 et al., 2001)(Fig. 5e).

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960 Direct export flux of *Trichodesmium* spp. blooms may also occur in cases where rapid (< 2 d)
961 bloom mortality occurs via a programmed cell death (PCD) ~~process that is induced under~~
962 ~~environmental stressors (e.g. Fe limitation, oxidative stress) or physiological status (stationary~~
963 ~~phase)~~ (Berman-Frank et al., 2004; Berman-Frank et al., 2007). PCD in *Trichodesmium* spp.
964 is ~~also~~ characterized by the loss of buoyancy (collapse of gas vesicles) and increased
965 production of TEP and aggregation leading to enhanced and massive vertical flux (Bar-Zeev
966 et al., 2013). A *Trichodesmium* spp. bloom that occurred outside the VAHINE mesocosms on
967 days 23-24 displayed mechanistic features of PCD including mass mortality within 24 h, loss
968 of gas vesicles, and high production of TEP (Spungin et al., In review, 2016). While we could

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969 not directly quantify the export flux as no sediment traps were deployed in the lagoon water
970 outside the mesocosms, the characteristics of the bloom, lack of grazer influence and the
971 demise of biomass suggests this would lead to high rates of export (Spungin et al., In review,
972 2016) as demonstrated in culture simulations (Bar-Zeev et al., 2013) (Fig 5c).

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974 4.2.2 DDN transfer to zooplankton

975 DDN transfer to zooplankton may either be direct through the ingestion of diazotrophs, or
976 indirect, i.e. mediated through the release of dissolved DDN by diazotrophs taken up by
977 heterotrophic and autotrophic plankton and subsequently grazed by zooplankton. During the
978 VAHINE experiment, the percent contribution of DDN to zooplankton biomass averaged 30
979 % (range = 15 to 70 %) (Hunt et al., Accepted), which is in upper range of values reported
980 from high N₂ fixation areas such as the subtropical north Atlantic (Landrum et al., 2011;
981 Mompean et al., 2013; Montoya et al., 2002a), the Baltic Sea (Sommer et al., 2006; Wannicke
982 et al., 2013b), and the pelagic waters off the New Caledonian shelf (Hunt et al., 2015).

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983 During VAHINE all four of the qPCR targeted diazotrophs (*Trichodesmium* spp., het-1, het-2,
984 UCYN-C) were found in zooplankton guts indicating a direct grazing of these four phylotypes
985 (Hunt et al., Accepted). Overall, the most frequently detected targets were het-1 (during P1;
986 17 to 180 *nifH* copies copepod⁻¹) and UCYN-C (during P2; 7 to 50 *nifH* copies copepod⁻¹), i.e.
987 the most abundant phylotypes encountered in the mesocosms during P1 and P2, respectively.
988 However, *Trichodesmium* spp. and het-2 were also detected at relatively high abundances in
989 copepod guts (~280 *nifH* copies copepod⁻¹) despite their low abundance in the mesocosms,
990 suggesting selective feeding and a possible top down control through zooplankton grazing for
991 these two phylotypes.

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992 Direct and efficient zooplankton grazing on UCYN-C was further substantiated by targeted
993 grazing experiments during VAHINE which consisted of ¹⁵N₂-labeled bottle incubations of
994 freshly collected zooplankton in the presence of natural phytoplankton assemblages. The ¹⁵N₂
995 label was taken up by the diazotroph in the incubation bottles and used as a marker of
996 zooplankton diazotroph ingestion and/or ingestion of non-diazotrophic plankton grown on
997 DDN. Zooplankton were highly ¹⁵N enriched after 72 h of incubation during the UCYN-C
998 bloom (P2), slightly enriched during P1 when DDAs dominated to diazotrophic community,
999 and not enriched at all when a *Trichodesmium* spp. bloom was encountered outside the
1000 mesocosms during P2 (Hunt et al., Accepted). This was a surprising finding given that het-1,
1001 and to a lesser extent *Trichodesmium* spp. were detected in copepod guts, and would suggest
1002 that UCYN-C are much more efficiently transferred to zooplankton compared to DDAs and

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1003 *Trichodesmium* spp. While we demonstrated direct grazing of zooplankton on *Trichodesmium*
1004 spp., DDAs and UCYN-C, further studies are required to quantify a more general contribution
1005 of direct and indirect transfer of DDN to zooplankton.

1006

1007 **5 Modelling as a tool to infer the fate of DDN and the role of N₂ fixation on** 1008 **productivity, food web structure and C export**

1009 Modelling has accompanied every stage of the VAHINE project. Mesocosm 1D-vertical
1010 simulations with the biogeochemical mechanistic Eco3M-MED model (Alekseenko et al.,
1011 2014), enriched with diazotrophs for the present study, and embedded in the Eco3M
1012 modelling platform (Baklouti et al., 2006), were utilized prior to the *in situ* experiments to aid
1013 in the scientific design of the experiment and in understanding the need and the optimal
1014 timing of the DIP enrichment. The biogeochemical model was first assessed using *in situ* data
1015 from the mesocosms and then applied to study the fate of DDN in the ecosystem (Gimenez et
1016 al., In review, 2016). Finally, one of the main strengths of the modelling tool lies in the
1017 opportunity that it offers to ~~deconvolute-separate~~ the different processes that are deeply
1018 interlinked. ~~This last facility is used~~ [Here we employed this here](#) to infer the role of N₂ fixation
1019 on productivity, food web structure, and C export. The simulation of the mesocosm
1020 experiment (including DIP enrichment) reported in Gimenez et al. (In review, 2016) hereafter
1021 referred to as the ‘REF’ simulation, and its main results relative to the fate of the DDN are
1022 summarized below.

1023

1024 At the end of the REF simulation (set at 25 days in the model), 33 % of the DDN was found
1025 in the diazotrophs, 43 % in the non-diazotroph organisms, 16 % in the DON pool, 3 % in the
1026 particulate detrital organic pool and 5 % in the traps, indicating that N₂ fixation efficiently
1027 benefited non-diazotrophic organisms and contributed to particle export. The model results
1028 substantiated the mass balance of N (Berthelot et al., 2015b) demonstrating that during the ~~10~~
1029 first 10 days of the experiment, planktonic organisms did not significantly benefit from the
1030 DDN and that DDN did not accumulate in the water column (was not transferred to non-
1031 diazotrophic plankton). After day 10, the DDN proportion increased in all the non-
1032 diazotrophic plankton groups, and simultaneously decreased in the non-living pools, although
1033 DON concentrations lagged decreasing only from day 13. This decrease in DDN proportion in
1034 the abiotic N pools is due both to the assimilation of mineral and organic nutrients by
1035 phytoplankton and heterotrophic prokaryotes, as well as to the sinking of the produced
1036 organic matter through aggregation processes.

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1037 The model results further showed that the fraction of DDN in the exported particulate matter
1038 increased from day 10 until the end of the simulation, consistent with the high e -ratio
1039 ~~determined by~~ (Berthelot et al., 2015b) during P2 (see above) and with the $\delta^{15}\text{N}$ -budget
1040 ~~performed by~~ (Knapp et al. (submitted)), emphasizing the higher contribution of N_2 fixation to
1041 export production during P2 compared to P1 (Gimenez et al., In review, 2016).

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1042 In the model, diazotrophs were assumed to release equal amounts of NH_4^+ and DON at a rate
1043 which increases non-linearly with the absolute and relative N contents of diazotrophs
1044 (Gimenez et al., In review, 2016). During P1, DDN accumulated in the DON pool (nearly up
1045 to 40% of the DDN generated from the beginning of the experiment ~~is~~ found in DON on
1046 day 13), whereas the proportion of DDN associated with NH_4^+ decreased rapidly from day 5
1047 as NH_4^+ was immediately used by heterotrophic bacteria and phytoplankton. The proportion
1048 of DDN associated with DON decreased later (i.e. during P2) when the inorganic N pool was
1049 depleted. The model results are consistent with the ^{15}N measurements from the NH_4^+ and
1050 DON pools, indicating that NH_4^+ was preferentially transferred to non-diazotrophic plankton
1051 compared to DON, which accumulated in the dissolved pool (Berthelot et al., In review,
1052 2016).

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1053 The model results were further validated in the distribution of the DDN among the biotic
1054 compartments. Small-size (pico- and nano-) phytoplankton, heterotrophic prokaryotes,
1055 heterotrophic nanoflagellates and ciliates were the main beneficiaries of DDN, as observed by
1056 the nanoSIMS studies (Berthelot et al., In review, 2016; Bonnet et al., 2016a). Small-size
1057 phytoplankton and heterotrophic prokaryotes were indeed the main consumers of NH_4^+ and
1058 labile DON (the model excludes DON uptake by large-size phytoplankton), and heterotrophic
1059 nanoflagellates and ciliates respectively feed on heterotrophic prokaryotes and small-size
1060 phytoplankton. These results therefore indicate that DDN ~~was mainly transited~~ transferred
1061 ~~predominantly~~ through pico-, nanophytoplankton, and ~~the actors of the~~ the microbial loop
1062 during the VAHINE experiment.

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1063
1064 Both the *in situ* and modelling work summarized in the previous sections demonstrate the
1065 important contribution and role of the diazotrophic communities to PP (non-diazotrophic) and
1066 BP, to zooplankton feeding, and eventually to C export.

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1067 To further assess the role of N_2 fixation ~~on~~ within the ecosystem, we used the REF simulation
1068 from Gimenez et al. (In review, 2016) and compared it to a new simulation in which we
1069 removed the N_2 fixation capability of diazotrophs (hereafter named 'NOFIX simulation'). The
1070 NOFIX simulation also included the following changes compared to the REF simulation to be

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1071 consistent with the new environmental conditions: (i) the initial relative N quotas of
1072 diazotrophs have been set to 25 % (instead of 100 % in the reference simulation, i.e. same
1073 value as the one used for non-diazotrophs). As the initial total N was identical to the one of
1074 the REF simulation, the N content of diazotrophs has been allocated to the detrital N
1075 compartment; (ii) all along the NOFIX simulation, only the detrital particulate compartment is
1076 allowed to sink at a constant rate of 0.7 m d⁻¹ (see Gimenez et al. (In review, 2016)), whereas
1077 in the REF simulation, this was also the case only until day 10 beyond which all the
1078 compartments were allowed to sink at a rate increasing with time, in order to mimic the
1079 observed increase in the particulate sinking flux due to TEP release and aggregation .

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1080 When comparing the REF and NOFIX simulations (Fig. 6), we note that the shapes of the PP
1081 and BP curves remain the same, showing an increase in PP and PB during P2 in both
1082 simulations. However, in the NOFIX simulation, the magnitude of PP and BP is reduced by
1083 2.5 and 1.5-fold respectively. Furthermore, according to the model, N₂ fixation fueled 43.5 %
1084 of PP and 8 % of BP during the 23 days of the simulated experiment. This

1085 ~~The fact that the resulting PP was reduced to a larger extent than the BP when N₂ fixation was~~
1086 ~~absent~~ does not necessarily mean that non-diazotrophic autotrophs benefit more from the
1087 DDN compared to heterotrophs as the DDN was nearly equally distributed between
1088 autotrophs and heterotrophs (and slightly higher in heterotrophs) (Gimenez et al., In review,
1089 2016). This higher effect on PP than on BP is derived from the fact that the diazotrophs
1090 themselves (and therefore a part of PP since only autotrophic diazotrophs were considered in
1091 the model) were strongly affected by their inability to fix N₂ as suggested by the far lower
1092 abundance of UCYN-C in the NOFIX simulation compared to the REF one (Fig. 6). This also
1093 explains why removing N₂ fixation first affected PP (~~around ~~~ day 10) and only later
1094 influenced compared to BP (~~around ~~~ day 15).

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1095 We further assumed that, apart from diazotrophs, the organisms mostly primarily influenced
1096 by the ~~absence~~ lack of N₂ fixation (in the simulation) should be ~~these~~ organisms that
1097 benefited the most from the DDN (i.e. in which the highest percentages of DDN have been
1098 calculated by the model (see Fig. 6 in ~~Gimenez et al. (2016)~~, Gimenez et al. (In review,
1099 2016)). These organisms include namely small (< 10 μm) phytoplankton, heterotrophic
1100 prokaryotes, heterotrophic nanoflagellates, and ciliates. ~~This was the case for s~~Small
1101 phytoplankton and heterotrophic bacteria were indeed influenced (Fig. 7), and to a lesser
1102 extent and later ~~for~~ heterotrophic nanoflagellates and ~~. This was also true for~~ ciliate
1103 abundance, but only until day 16. After day 16, ciliate abundance was slightly higher (<5 %
1104 between day 16 and 23) ~~higher~~ in the NOFIX simulation compared to the REF one, resulting

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1105 predominantly from a top-down effect due to increased copepod predation in the NOFIX
1106 simulation from day 10 to day 23 (results not shown).

1107 Our model did not include DDAs and did not allow the uptake of DON by large
1108 phytoplankton (i.e. diatoms). Thus, the DDN content in diatoms, and therefore in
1109 mesozooplankton, was probably slightly underestimated by the model in the REF simulation
1110 (Gimenez et al., In review, 2016) compared to *in situ* data (Hunt et al., Accepted). As a result,
1111 large phytoplankton and mesozooplankton abundances were nearly similar in the REF and
1112 NOFIX simulations (not shown). Hence, apart from ciliates (~~which~~ whose mortality also fuels
1113 the detrital particulate compartment as ~~for~~ large phytoplankton and mesozooplankton), the
1114 organisms that mostly benefited from the DDN were the small organisms, ~~the~~ which mortality
1115 ~~of which~~ fuels the dissolved organic pool.

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1116
1117 How does N₂ fixation impact C export? Absence of N₂ fixation (NOFIX simulation) reduced
1118 export by 30 % on day 23 compared to the REF simulation (Fig. 8). This difference in C
1119 export reaches 50 % when the simulation duration is extended until day 35 (not shown).
1120 These results indicate that N₂ fixation and the subsequent new production promotes C export
1121 to depth as the experimental VAHINE results demonstrated (Berthelot et al., 2015b; Knapp et
1122 al., 2015).

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1123 It is likely that during the experiment, TEP release favored aggregation and accumulation of
1124 particles and subsequently enhanced vertical flux from the different compartments in the
1125 water column. To represent the latter phenomenon, we considered in the model that 10 % of
1126 the living and non-living compartments were allowed to sink after day 10 (see Gimenez et al.
1127 (2016) for more details). Since this extra aggregation is mainly attributable to diazotrophs, it
1128 was not represented in the NOFIX simulation. However, we ran a third simulation (not
1129 shown) to further analyze the excess of C export in the REF simulation as compared to the
1130 NOFIX one (Fig. 8). This third simulation is intermediate between the REF and the NOFIX
1131 simulations in that sense that only the N₂ fixation capability by diazotrophs is removed (but
1132 aggregation processes are still represented). This simulation indicated that C export is nearly
1133 equal to that of the REF simulation after 25 days (they differ by only 2.9 %), thereby
1134 suggesting that during the 25 first days, the suppression of N₂ fixation does not significantly
1135 impact carbon export fluxes. This further suggests that the higher C export in the REF
1136 simulation during P2 (Fig.8) is mainly due to aggregation processes mediated by diazotrophs-
1137 derived TEP release and the subsequent export of diazotrophs (Berman-Frank et al., 2016;
1138 Bonnet et al., 2015a). However, beyond day 25, the difference in C export between the REF

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1139 ~~and the third simulation increases up to 25% on day 35. In other words, the N₂ fixation~~
1140 ~~process per se (by supporting PP and BP fluxes) contributes more and more to the enhanced C~~
1141 ~~export as N₂ fixation fluxes increase. Hence, on day 30, N₂ fixation supports ~50 % of the~~
1142 ~~excess C export observed between the REF and the NOFIX simulations, the remaining still~~
1143 ~~being attributed to aggregation processes.~~

1144 ~~It is likely that a combination of chemical and physical properties of the water~~
1145 ~~combined with the enrichment in DOM increased stickiness and aggregate properties~~
1146 ~~cause further accumulation, induced aggregation and accumulation of particles and a~~
1147 ~~subsequently enhanced, and enhance vertical flux from the different compartments in~~

1148 ~~the water column. To represent the latter phenomenon, we considered in the model~~
1149 ~~that 10 % of the living and non-living compartments are allowed to sink after day 10~~
1150 ~~(see Gimenez et al. (2016) for more details). We ran a third simulation (not shown),~~

1151 ~~to further analyze the excess of C export in the REF simulation as compared to the~~
1152 ~~NOFIX one. Herein which the N₂ fixation capability by diazotrophs is still was removed~~
1153 ~~but in which the aggregation processes were represented (in the same way as in the~~

1154 ~~REF simulation), has been run in order to further analyze the excess of C export in~~
1155 ~~the REF simulation as compared to the NOFIX one (Fig. 8). This third simulation~~
1156 ~~indicated that C export is nearly equal to that of the REF simulation after 25 days~~

1157 ~~(they differ by only 2.9 %), with 25 % difference reached on day 35. This suggests~~
1158 ~~that the higher C export in the REF simulation during P2 is mainly due to~~
1159 ~~aggregation processes mediated by diazotrophs-derived TEP release and the~~

1160 ~~subsequent export of diazotrophs (Berman-Frank et al., 2016; Bonnet et al.,~~
1161 ~~2015a). A third simulation (not shown) in which the N₂ fixation capability by diazotrophs is~~
1162 ~~still removed but in which the aggregation processes were represented (in the same way as in~~

1163 ~~the REF simulation) indicated that C export is nearly equal to that of the REF simulation after~~
1164 ~~25 days (they differ by only 2.9 %), with 25 % difference reached on day 35. This suggests~~
1165 ~~that the higher C export when N₂ fixation is active occurs initially due to aggregation~~

1166 ~~processes mediated diazotrophs-derived TEP release and the subsequent export of diazotrophs~~
1167 ~~(Berman-Frank et al., 2016; Bonnet et al., 2015a). Moreover, it is likely that increased~~

1168 ~~stickiness and aggregate properties also cause further accumulation, aggregation, and~~
1169 ~~enhanced vertical flux from the different compartments in the water column. To represent the~~
1170 ~~latter phenomenon, we considered that 10 % of the living and non-living compartments are~~

1171 ~~allowed to sink after day 10 in the model (see Gimenez et al. (2016) for more details). In a~~
1172 ~~second step however, the N₂ fixation process per se (by supporting PP and BP fluxes)~~

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1173 ~~contributes more and more to the enhanced C export as N₂ fixation fluxes increase. Hence, on~~
1174 ~~day 30, N₂ fixation supports ~50 % of the excess C export observed between the REF and the~~
1175 ~~NOFIX simulations, the remaining still being attributed to aggregation processes.~~

1176 To conclude, N₂ fixation has a significant impact on both direct and indirect C export via
1177 diazotroph fueling of non-diazotrophic plankton as well as via aggregation processes. The
1178 model provides a lower limit of the major role played by N₂ fixation on C export due to an
1179 underestimate of the DDN content in diatoms, and in mesozooplankton. Finally, this study
1180 also points the need of further investigation on aggregation processes in relation with TEP
1181 release and its representation in models since its influence on C export may be of the same
1182 order of magnitude as the N₂ fixation process per se.

1183

1184 **6 Conclusions and future work**

1185 The VAHINE project provided unique opportunities to study and compare the fate of N₂
1186 fixation associated with different diazotrophs in the marine environment. The results showed
1187 that when the diazotroph community was dominated by DDAs, the DDN remained within the
1188 symbiotic associations, was poorly transferred to the non-diazotrophic phytoplankton and
1189 heterotrophic prokaryotes, yet ~~can~~ could be transferred directly to zooplankton through
1190 grazing. The project results further substantiated previous data showing rapid export to depth
1191 of the recently fixed N₂ by DDAs (Karl et al., 2012). An opportune bloom of UCYN-C during
1192 the VAHINE project demonstrated that when UCYN-C dominated the diazotroph community,
1193 ~ 25 % of the DDN was quickly (24 h) transferred to the planktonic food web through the
1194 release of DON and NH₄⁺ to the dissolved pool. These additional N sources were
1195 subsequently transferred to zooplankton, both directly (through the grazing of UCYN-C) and
1196 indirectly through the grazing of plankton grown on DDN from UCYN-C. Moreover, the
1197 VAHINE data explicitly revealed that when UCYN-C dominated the diazotroph community,
1198 the efficiency of the system to export POC relative to PP (*e*-ratio) ~~was~~ higher than when
1199 DDAs dominated. This export is both direct, through the sinking of small (5.7±0.8 μm)
1200 UCYN-C cells aggregated into large (100-500 μm) particles having high sinking rates, and
1201 indirect through the sinking of plankton benefitting from the enriched source of DDN. Future
1202 projects should extend the investigation of DDN export below the photic layer in the open
1203 ocean (~70-150 m in the oligotrophic ocean) to confirm the process study obtained during
1204 VAHINE in mesocosms in an experimental 15 m-depth water column. In particular, are the
1205 aggregation processes of UCYN also observed in the open ocean? Although technically and
1206 logistically challenging, this feat may be accomplished through locating a research vessel in a

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1207 ID structure (cyclonic eddy harboring high UCYN abundances for example) where horizontal
1208 advection is reduced and sediment traps are deployed to study the biological and
1209 biogeochemical characteristics of the photic zone for one to two weeks.

1210 The VAHINE project also provided a unique opportunity to compare the transfer efficiency of
1211 DDN from UCYN and *Trichodesmium* spp. to the different compartments of the planktonic
1212 food web, and revealed that the main beneficiaries of the DDN depend on both the
1213 physiological status (e.g. nutritionally balanced, stationary or decline phase) and the type of
1214 diazotroph. When *Trichodesmium* spp. bloom decay they release large amounts of NH_4^+ and
1215 mainly support diatom growth, indicating a large potential of indirect organic matter export
1216 during/after *Trichodesmium* spp. blooms. This is further substantiated by the study of PCD
1217 indicating a rapid direct export of *Trichodesmium* spp. itself but further studies are needed in
1218 open ocean *Trichodesmium* spp. blooms to extrapolate our results to the field.

1219 NH_4^+ appears to be the main form of DDN transferred to non-diazotrophic plankton. In future
1220 studies, it would be necessary to refine the chemical composition of DON released by
1221 different diazotrophs to assess its lability as a function of the diazotrophs involved in N_2
1222 fixation and the stage of the bloom. It would also be informative to explore the amount and
1223 chemical composition of released DOC and better study the potential of diazotrophs to
1224 stimulate heterotrophs and their subsequent impact on the ocean metabolic balance.

1225 Finally, in the future ocean, some diazotrophs such as *Trichodesmium* spp. (Hutchins et al.,
1226 2007; Levitan et al., 2007) and UCYN-B (Fu et al., 2008) (no study is available on UCYN-C)
1227 may develop extensively under high temperature and $p\text{CO}_2$ conditions (Dutkiewicz et al.,
1228 2015), while others such as UCYN-A would not be affected (Law et al., 2012). The results
1229 from the VAHINE project revealed that the diazotroph community composition ~~has a can~~
1230 ~~profound~~ impact ~~in the structuring the~~ planktonic food web ~~structure and composition~~ in the
1231 surface ocean, and ~~in also affects~~ ~~the the~~ efficiency of particulate matter export to depth. Thus,
1232 current and predicted global changes require further knowledge and understanding of the fate
1233 and implications of changing scenarios of N_2 fixation in the future oceans.

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1278 **Figure legends.**

1279

1280 **Figure 1.** Study site of the VAHINE experiment. Location map of New Caledonia in the
1281 Southwestern Pacific (a), Map of the Noumea lagoon showing the location of mesocosms at
1282 the entrance of the lagoon, 28 km off the coast (b).

1283

1284 **Figure 2.** View of the mesocosms from above (a), from the seafloor (b) and view of the
1285 sediment traps that collect sinking particles (c) (Photos credits: J.M. Boré and E. Folcher,
1286 IRD).

1287

1288 **Figure 3.** Evolution of sea surface temperature ($^{\circ}\text{C}$) (a), NO_3^- ($\mu\text{mol L}^{-1}$) (b), DIP ($\mu\text{mol L}^{-1}$)
1289 (c), Chl a ($\mu\text{g L}^{-1}$) (d), N_2 fixation rates ($\text{nmol N L}^{-1} \text{d}^{-1}$) (e), PON concentrations ($\mu\text{mol L}^{-1}$)
1290 (f), DON concentrations ($\mu\text{mol L}^{-1}$) (g) and PON export ($\mu\text{mol Ld}^{-1}$) (h) over the 23 days of
1291 the VAHINE mesocosm experiment. Lines represent the average of the three mesocosms and
1292 shaded areas represent the measured min and max values.

1293

1294 **Figure 4.** Upper panel: Diazotroph community composition in the VAHINE mesocosm
1295 experiment during the experimental period. *nifH*-based abundances were summed for each
1296 sampling day to determine the percent contribution to the total diazotroph community from
1297 each major phylotype (data from Turk-Kubo et al. (2015)). Bottom panel: simplified
1298 evolution of the major standing stocks, rates and plankton abundances measured during P1
1299 (days 5 to 14) and P2 (days 15 to 23). ~~Protocols for each parameter measurements are in the~~
1300 ~~mesocosms as~~ described in Berthelot et al. (2015), Bonnet et al. (2016a,b), Van Wambeke et
1301 al., (2016), Berman-Frank et al., (2016), ~~Hunt et al. (2016), LeBlanc et al. (2016), Turk-~~
1302 ~~Kubo et al., (2015) and Hunt et al., (2016) Spungin et al. (2016) and Wanvanbeke et al.~~
1303 ~~(2016)~~. Squares are represented in green when a significant ($p < 0.05$) increase was observed
1304 between each period (i.e. between P0 and P1 or between P1 and P2, Kruskal-Wallis test,
1305 $\alpha = 0.05$), in red when a significant ($p < 0.05$) decrease was observed and in grey when no
1306 significant change was observed between the different periods.

1307

1308 **Figure 5.** Summary of the simplified pathways of the potential DDN transfer in the first
1309 trophic level of the food web and potential of direct *versus* indirect export of particulate
1310 matter for DDAs (a), UCYN-C (b) and *Trichodesmium* (c). DDN transfer data from (Bonnet
1311 et al., Accepted; Bonnet et al., 2016a)

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Figure 6. Evolution of PP ($\mu\text{mol C L}^{-1} \text{d}^{-1}$) (a) and bacterial production ($\text{ng C L}^{-1} \text{h}^{-1}$) in the REF simulation (black-blue line) and the NOFIX simulation (black line) (i.e. when the N_2 fixation process is removed).

Figure 7. Evolution of plankton abundances (cells L^{-1}) in the REF simulation (blue line) and the NOFIX simulation (black line) (i.e. when the N_2 fixation process is removed). TRI: *Trichodesmium* spp., UCYN: UCYN-C, BAC: heterotrophic bacteria, PHYS: small phytoplankton, HNF: heterotrophic nanoflagellates.

Figure 8. Evolution of C content collected in the mesocosm particle traps (mmol C) in the REF simulation (blue line) and the NOFIX simulation (black line) (i.e. when the N_2 fixation process is removed).

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