Reply to the reviewers, on the behalf of all co-authors

General reply to the reviewers

We would like to greatly thank the referees for their relevant comment on the recovery of non-specific plastid sequences, and for their helpful spelling and grammar corrections.

The general comments of both reviewers concern the English language and the recovery of plastid sequences. We agree with all comments and adressed them as advised. Concerning the English language, all spelling and grammar errors noticed by reviewers #1 and #2 were adressed and some sections were re-written to improve the reading of the manuscript. Concerning the recovery of plastid sequences, the Referee #2 suggested that it was the result of non specific amplification, and that "the only way to know the obtained 16S rRNA sequences have an identical sequence in the Nitro821 primer region is to find a sequence identical to theirs in Genbank but obtained with a different primer pair that is further toward the end of the 16S rRNA gene than the Nitro821 primer".

Genbank closest relative sequences of each plastid groups have been looked for and 4 to 7 mismatches with the Nitro821 oligonucleotide were detected (see Table a). Biegala and Raimbault (2008) have demonstrated that the non-UCYN strains PCC73106 and PCC6803, which have 3 and 4 mismatches with Nitro821 respectively (see Table a), are not hybridized by the Nitro821 probe. Moreover, Mazard et al. (2004) showed that sequences having more than 1 mismatche with Nitro821 are not PCR amplified during Nitro821/Cya359 PCRs. So far, only the freshwater cyanobacteria *Planktothrix* has 1 mismatche with the Nitro821 oligonucleotide, however this cyanobacteria is unlikely to be detected in the marine environment, (Mazard et al., 2004).

We thus agree with Referee #2 that the plastid sequences are the result of non-specific amplifications made possible by the use of nested PCR on 16S rDNA gene. Consequently, the discussion on plastids is not correct and has removed entirely.

Table a. Number of mismatches between the Nitro821 oligonucleotide and 16S rDNA target sequences from plastids and two cyanobacteria.

Phyloge-netic group	Nb of clo-nes	Iden-tity (%)	Probe, target, or closest relative	Sequence*					
			Nitro821	3'- CTT	TGA	TCC	ACA	CCG	AAC - 5'
			Target	5'- GAA	ACT	AGG	TGT	GGC	TTG - 3'
		99	Unc. bacterium clone S25_1306 (EF574962)	. . T	. . .	. . .	. . .	T . .	G . A
Group 2	5	91	*Ochromonas distigma* RCC21 (AY702136)	. . T	. . .	. . A	. . .	T . T	. GT
Group 3	1	98	Unc. cyanobacterium SHAB462 (GQ348575)	. . T	. . .	. . A	. . .	T . T	. GT
Group 4	1	91	Unidentified eukaryote OM270 (U70723)	. . T	. . .	. . A	. . .	T . .	G . A
Group 5	1	96	Unidentified eukaryote OM270 (U70723)	. . T	. . .	. . A	. . .	T . .	G . A
Group 6	3	87	*Crustomastix stigmatica* (FN563093)	. . .	. . .	. . .	. . G	T .G	GGA
Group 7	3	88	*Crustomastix stigmatica* (FN563093)	. . .	. . .	. . .	. . G	T .G	GGA
Group 8	2	94	*Dolichomastix tenuilepis* (FN563094)	A. .	. . .	. . .	. . .	T .G	GCT
Group 9	1	99	Unc. bacterium (AB307974)	. . T	. . .	. . .	. A .	TTA	A . T
			Gloeocapsa sp. strain PCC 73106	. . T	. . .	. . .	C . .	A . .	. . .
			Synechocystis sp. strain PCC 6803	. . T	. . .	. . .	. . .	T . T	C . .

** dots indicate bases identical to those of the target sequence.*

The paper has been modified as follow:

- **Material & Methods, section 2.4. PCR and cloning**: the nested protocol for 16S rDNA amplification has been removed.
- **Results, section 3.3. Diazotrophs species richness**: results concerning 16S rDNA phylogenetic analyses have been modified as follow: "UCYN specific 16S rDNA amplification was obtained only from station A and all the sequences were affiliated to UCYN-A". Moreover, according to the suggestion of Referee #2, we highlighted the discovery of the new marine group of *Bradyrhizobium* by adding a figure illustrating the percentage of each group of diazotrophs at station A, B, and C (Figure 7, see below).

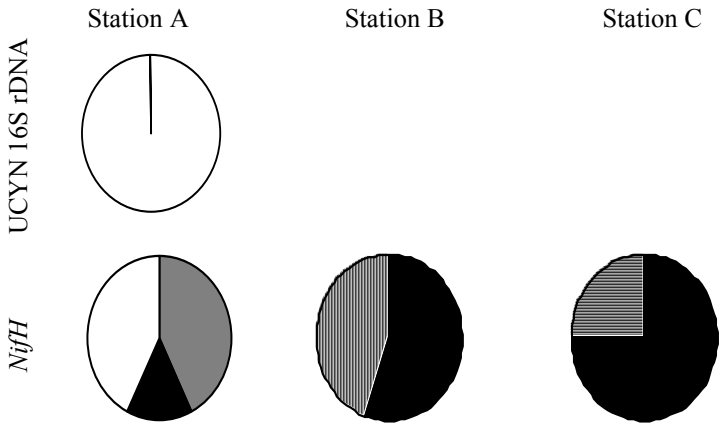

Fig. 7

Fig. 7. *Percentage of sequences from UCYN specific 16S rDNA and NifH clone libraries from station A, B, and C. Colors indicate phylogenetic groups from figure 5 and 6 in which sequences were recovered: UCYN-A group 1' (white); Bradyrhizobium group 2' (black); Bradyrhizobium group 3' (grey); rhizobia group 4' (horizontal lines); γ-proteobacteria group 5' (vertical lines).*

- **Discusssion: section 4.2. UCYN species richness recovery on 16S rDNA**: The discussion on plastids recovery has been removed and replaced by a discussion on the recovery of UCYN-A at station A and on the absence of positive 16S rDNA PCR amplification at station B and C, due to the too low cells concentration.
- **Fig 5:** all plastid sequences have been removed
- **Table 2:** all plastid sequences have been removed

Specific comments:

PG 3- LINE 6- COMPARED TO REDFIELDS (N:P = 16:1)... this has been corrected by "compared to Redfield", as proposed by Referee #2.

PG 3- LINE 17- CONTROLLED... this has been corrected.

PG 3- LINE 18- SAÑUDO-WILHELMY ET AL., 2001)... This mistake has been corrected.

PG 3- LINE 25 ...THAN LARGER ONES (THEIR IS A MEPS 2005 BY FALCON ET AL THAT WORKS ON P ADQUISITION BY UNICELLULAR FIXERS). The mistake has been corrected, and the reference to Falcón et al. (2005) has been added.

PG 4- LINE 25- ... WERE LOW ALONG A SEASONAL CYCLE (4.6 CELLS ML-1) COMPARED TO ABUNDANCE ESTIMATES FROM OTHER OCEANIC BASINS (CITE RELEVANT REFERENCES)...,EXCEPT FOR SUMMER ABUNDANCES THAT REACHED 1900-5300 .... The following references have been added: "Their concentrations were low along a seasonal cycle (4.6 cell ml^{-1}) compared to abundance estimates from other oceanic basins (reviewed in Le Moal and Biegala, 2009; Moisander et al., 2010), except for summer abundances that reached 1900-5300 cell ml^{-1}**."**

PG 5-LINE 3- (Q-PCR) OF NIFH COPIES... this has been corrected.

PG 5- LINES 1-24, RE WRITE, MIXED IDEAS AND MAJOR ENGLISH GRAMMAR ISSUES. The paragraph dedicated to molecular tools used to study the concentration and the species richness of diazotroph has been rewritten (cf. revised manuscript).

PG 7- LINE 6, CROCOSPAHERA WATSONII WH8501 WAS... We do not agree with this comment as the accurate spelling is "*Crocosphaera watsonii* WH8501".

PG 7-LINE 25, CELLS WERE SUBSEQUENTLY... ACCORDING TO BIEGALA AND RAIMBAULT... The sentence has been modified.

PG 11-LINE 1, FROM SEVEN TO 1 CELL ML-1... this has been corrected.

PG 13-LINE 4- REVEALED A VERY... this has been corrected.

PG 14- RE WRITE, LINES 19-28... IDEAS ARE MIXED, GENERAL ENGLISH USAGE... This paragraph on plastid sequences recovery has been removed (cf. General comments).

PG 14- LINE 14 ON... We do not understand this comment.

PG 15-LINE IS 18...THIS RELEVANT? We agree, the sentence is not necessary and has been removed.

PG 15- LINE 22- RE WRITE... This paragraph on plastid sequences recovery has been removed (cf. General comments).

PG 16- LINE 14- THE R. GIBBA SYMBIONT HAS...., WHILE IN THIS STUDY NIFH GENE WAS NOT AMPLIFIED...CONTRADICTORY, NOT CLEAR. This paragraph on plastid sequences recovery has been removed (cf. General comments).

PG 17- LINE 18- FOR TWO THIRDS... this has been corrected.

PG 18 NEEDS AN IN DEPTH REVISION OF GRAMMAR... LAST SENTENCE- CLARIFY SINCE THE MEDITERRANEAN IS KNOWN FOR IRON DEPOSITION FROM THE SAHARA. Many mistakes have been corrected, according comments of Referee #2. The sentence on iron has been deleted on the request of Referee #2.

PG 21-LINES 3- ARE NOT EXPRESSED AS HYPOTHESIS... We understand the need of this paragraph to be expressed as hypothesis, that is why we used many expression of conditional such as: "We hypothesis that", "may be explained"…etc in the earlier version of the manuscript.

THE CONCLUSSION THAT PLASTID SEQUENCES RECOVERED WITH UCYN PROBE REGARDING THE SEQUENCES RECOVERED IS OVER DIMENSHIONED.OBVIOUSLY, YOU WOULD HAVE A POSSITIVE SIGNAL FOR THE UCYN PROBE WITH PLASTIDS SIMPLY DUE TO THEIR CLOSE PHYLOGENETIC AFFILITION. THE NULL EXPLANATION IS THAT NO-UCYN WERE PRESENT IN THE SAMPLES, AND ONLY PLASTIDS WERE SEEN. Please Cf the reply in general comments.