Nitrogen fixation and diffusive fluxes in the upwelling region off NW Iberia

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ABSTRACT

The classical paradigm about marine N_2-fixation establishes that this process is mainly constrained to oligotrophic tropical and subtropical regions and attributed mostly to the cyanobacterium *Trichodesmium*. However, the development of molecular techniques led to the discovery of a larger variety of marine diazotrophs, which extends the range of environments where N_2-fixation may be relevant. Between February 2014 and December 2015 we carried out 16 cruises in the upwelling ecosystem off NW Iberia with the following goals: 1) to quantify the magnitude of N_2-fixation, 2) to investigate its biogeochemical role as mechanism of new nitrogen supply, and 3) to identify and quantify the main diazotrophs in the region under contrasting hydrographic regimes. Our results indicate that the magnitude of N_2-fixation in this region is comparable to the lower-end of rates described for subtropical regions. All the N_2-fixation activity was detected in the smaller-sized (<10μm) fraction. The comparison with nitrate diffusive fluxes reveals the minor role of this process (<1%) as a mechanism of new nitrogen supply into the productive euphotic layer. Results obtained through phylogenetic analyses by Illumina® (NGS) show that the composition of the diazotrophic community presents a seasonal variability depending on hydrographic conditions. Additional experiments carried out in the field and in the lab demonstrate that ^{15}N-labeled contaminants included in some commercial $^{15}N_2$ stocks are assimilable by non-diazotrophs organisms. This could result in an up to 15-fold overestimation of N_2-fixation rates. Overall, our findings support the emerging view that mesotrophic regions should be considered in global budgets of marine N_2-fixation.

INTRODUCTION

Molecular nitrogen (N_2) is the most abundant form of nitrogen (N), however only a limited, but diverse, number of bacteria and Methanotrophic *Archaea* can use this reservoir through the process named N_2 fixation [1]. Thus, N is the main limiting nutrient in both marine and terrestrial ecosystems [2]. This biological process is the main mechanism that supplies bioavailable N in the oceans and it has a key role as controlling factor of the primary productivity, the carbon cycle and the climate [3]. Traditionally, N_2 fixation was mainly attributed to the colonial filamentous cyanobacterium of the genus *Trichodesmium* that inhabit oligotrophic tropical and subtropical regions of the oceans. The discovery of other groups of marine diazotrophs evidenced that the range of environments where N_2 fixation may be relevant is broader than it was originally thought [4]. Recent studies demonstrate the activity of diazotrophs in N enriched environments [5,6,7,8], where up to now it was considered insignificant. To our knowledge, only two previous studies have investigated planktonic N_2 fixation rates and its relevance in the upwelling region off NW Iberia [6,8].

A recent study showed that ^{15}N-labeled contaminants included in some commercial $^{15}N_2$ stocks are assimilable by non-diazotrophs organisms. These units are used, on a regular basis, to estimate N_2 fixation rates from incubations of environmental samples, by monitoring the incorporation of isotopically labeled $^{15}N_2$ into organic matter [9]. In this study we analyzed data collected in the upwelling ecosystem off NW Iberia in order to: 1) quantify the magnitude of N_2-fixation, 2) investigate its biogeochemical role as mechanism of new nitrogen supply, and 3) identify and quantify the main diazotrophs in the region.

MATERIAL AND METHODS

In the framework of the NICANOR project 16 samplings were carried out in the region off NW Iberia. Size-fractionated (>10 μm and <10 μm) N_2 fixation rates were measured using the $^{15}N_2$ uptake technique [10]. Measurements of dissipation rates of turbulent kinetic energy (ε) were carried out using a microstructure profiler (MSS, [11]). Vertical diffusivity (Kz) was calculated from ε and Brünt Väissälä frequency (N) [12]. Nitrate diffusive supply was computed as the product of averaged Kz across the nitracline and the nitrate gradient for the same depth interval. Size-fractionated primary production was estimated from simulated in situ ^{14}C-uptake incubations [13]. Size-fractionated chlorophyll *a* concentration was

determined using the spectrofluorometric method [13]. The concentration of nitrate and nitrite was determined with a Segmented Flow Analyser. Diazotrophic community composition was investigated by amplifying a partial *nifH* fragment using degenerate primers [14], and PCR amplicons were cloned and sequenced by Illumina®.

RESULTS AND DISCUSSION

The sampling station is located in a temperate region under the influence of coastal upwelling. Between February 2014 and December 2015 we sampled the characteristic seasonal hydrographic features in this region.

Depth-integrated N_2 fixation rates, that were attributed to the <10 µm fraction, ranged between ca. 0.3 and 15 µmol m^{-2} d^{-1} (see Fig.1).

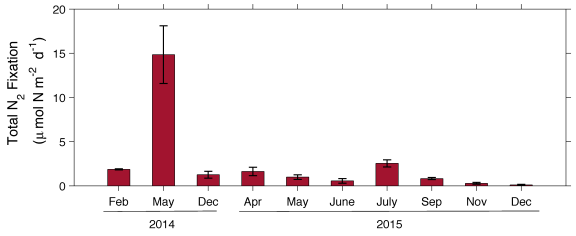

Fig. 1. Depth-integrated (from surface to 40 m depth) N_2 fixation rates estimated during the NICANOR cruises in the upwelling region off NW Iberia.

The highest N_2 fixation rates occurred in May 2014, coinciding with the highest chlorophyll *a* concentration. Our rates are comparable to the range previously reported by Benavides et al. in the same region [6], but up to 4 orders of magnitude lower than those described in [5,7] in the western of English Channel and in the North American Mid-Atlantic continental shelf, respectively. N_2 fixation rates at 70 m depth, mainly attributed to heterotrophic diazotrophs by *nifH* gene sequencing, were similar to those from shallower depths.

Analysis by NGS technology (Illumina®) of *nifH* genes revealed that the composition of the diazotrophic community varied depending on the prevailing hydrographic conditions. Overall, most of the sequences belong to the unicellular cyanobacteria Group A (UCYN-A or *Candidatus* Atelocyanobacterium thalassa), followed by bacteria from Phylum Firmicutes and γ-Proteobacteria.

The comparison of N_2 fixation rates and nitrate diffusive fluxes showed that diazotrophy, which represents < 1% of the N supply into euphotic zone, is a minor N input in this system.

Additional lab and field experiments showed that ^{15}N-labeled contaminants included in some commercial $^{15}N_2$ gas stocks are assimilable by non-diazotrophs organisms. This could result in an up to 15-fold overestimation of the N_2 fixation rates estimated in the field.

In conclusion, biological N_2 fixation has been proved in the study area by $^{15}N_2$ assimilation activity and the presence of *nifH* in various phylogenetic groups, supporting the emerging view that mesotrophic regions should be considered in global budgets of marine N_2 fixation.

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