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Ocean acidification impacts on nitrogen fixation in the coastal western Mediterranean Sea

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ABSTRACT

The effects of ocean acidification on nitrogen (N_2) fixation rates and on the community composition of N_2-fixing microbes (diazotrophs) were examined in coastal waters of the North-Western Mediterranean Sea. Nine experimental mesocosm enclosures of ~50 m^3 each were deployed for 20 days during June–July 2012 in the Bay of Calvi, Corsica, France. Three control mesocosms were maintained under ambient conditions of carbonate chemistry. The remainder were manipulated with CO_2 saturated seawater to attain target amendments of pCO_2 of 550, 650, 750, 850, 1000 and 1250 µatm. Rates of N_2 fixation were elevated up to 10 times relative to control rates (2.00 ± 1.21 nmol $L^{-1}d^{-1}$) when pCO_2 concentrations were >1000 µatm and pH_T (total scale) < 7.74. Diazotrophic phylotypes commonly found in oligotrophic marine waters, including the Mediterranean, were not present at the onset of the experiment and therefore, the diazotroph community composition was characterised by amplifying partial *nifH* genes from the mesocosms. The diazotroph community was comprised primarily of cluster III *nifH* sequences (which include possible anaerobes), and proteobacterial (α and γ) sequences, in addition to small numbers of filamentous (or pseudo-filamentous) cyanobacterial phylotypes. The implication from this study is that there is some potential for elevated N_2 fixation rates in the coastal western Mediterranean before the end of this century as a result of increasing ocean acidification. Observations made of variability in the diazotroph community composition could not be correlated with changes in carbon chemistry, which highlights the complexity of the relationship between ocean acidification and these keystone organisms.

1. Introduction

The impact of 250 years of industrial activity is now being detected throughout our environment at scales that range from cellular to regional and even global scales. The change in atmospheric carbon dioxide (CO_2) from ~280 parts per million (ppm) in pre-industrial times to ~399 ppm in 2014 has impacted the Earth system on several scales, not least of which are the warming of the atmosphere and the oceans as a result of an enhanced greenhouse effect (IPCC, 2013). The oceans and atmosphere are intimately linked so that changes to the partial pressure of atmospheric CO_2 result in proportional changes in dissolved CO_2 in the marine environment. As a result of this, the rise of global temperatures has been buffered by the exchange of approximately ~26% of anthropogenic CO_2 into the oceans (Le Quéré et al., 2014) and it is this condition that has resulted in a profound change to ocean carbonate chemistry and the phenomenon of ocean acidification (OA) (Raven et al., 2005). As a consequence, surface seawater pH is on average ~0.1 units lower than it was prior to the industrial revolution, which equates to an increase in acidity of 26%. Earth system models project a global additional decrease in pH by 2100 ranging from 0.06 to 0.32 units (15–110% increase in acidity) depending on our future CO_2 emissions (Ciais et al., 2013). Elevated oceanic partial pressure of CO_2 (pCO_2) and the subsequent decrease in pH will have direct and indirect impacts on microbial nutrient cycling and carbon fixation which may fundamentally alter current

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biogeochemical cycles (Hutchins et al., 2009).

Nitrogen (N_2) fixation is a critical process in the biogeochemical cycling of elements in sub-tropical and tropical, nutrient poor waters (Carpenter and Capone, 2008) which has an equivocal response to OA. Research efforts into the sensitivity of diazotrophic activity to OA have largely been focused on *Trichodesmium*. The first reports showed increased rates of N_2 fixation with partial pressures of CO_2 between 750 and 1250 μatm relative to ambient conditions (Levitan et al., 2007; Barcelos E Ramos et al., 2007; Hutchins et al., 2007; Kranz et al., 2009, 2010). These experimental investigations were performed under laboratory conditions using a cultured organism, and most of these experiments were performed using replete nutrient conditions. Hutchins et al. (2007) performed experiments under enriched and limiting conditions of phosphorus (P) to find that N_2 fixation rates were stimulated by higher levels of CO_2 even in cultures experiencing severe P limitation, despite P being one of the two nutrients most likely to limit N_2 fixation. Spungin et al. (2014) found that P limitation actually led to an enhancement of the OA stimulation of N_2 fixation by Trichodesmium. It would appear that limiting quantities of P might enhance diazotrophy, Shi et al. (2012) found that N_2 fixation rates of *Trichodesmium* were impaired under conditions of iron depletion. Fu et al. (2008) performed similar studies on the unicellular cyanobacterium *Crocosphaera watsonii* to find that under iron replete conditions N_2 fixation rates were enhanced at a pCO_2 of 750 μatm, compared to iron deplete conditions where no effect was observed. A negative impact of OA was also recorded, in *Nodularia spumigena,* a heterocystous diazotroph common to the Baltic sea (Czerny et al., 2009), where cell division rates and nitrogen fixation rates were reduced at CO_2 levels up to 731 ppm. Results from this small number of laboratory studies imply that N_2 fixation can be stimulated by OA, but that there may be a relationship with the nutrient regime and particularly the bioavailable iron concentration (Fu et al., 2008; Shi et al., 2012), and that this may vary between different diazotrophic organisms.

The available evidence of OA impacts on natural communities of diazotrophs is even more limited. Evidence presented by Hutchins et al. (2009) and Lomas et al. (2012) showed that natural populations of *Trichodesmium* in the Atlantic Ocean were stimulated by increases in CO_2 in a similar manner to those in culture. In contrast, Gradoville et al. (2014) found no evidence, during 3 cruises and 11 experiments in the North Pacific, of enhanced N_2 fixation by *Trichodesmium* under elevated levels of CO_2 and further, that there was no change from this under altered conditions of phosphorus, iron or light. Similarly, Law et al. (2012) and Böttjer et al. (2014) recorded no relationship between CO_2 and N_2 fixation for CO_2 amendments up to 750 and 1100 μatm respectively for natural diazotroph communities dominated by unicellular cyanobacteria in the North and South Pacific.

The Mediterranean is a semi-enclosed sea, which is oligotrophic in nature and, due to its short ventilation period and dense urbanisations close to the coastal areas, is susceptible to anthropogenic driven influences (The Mermex Group et al., 2011). Recent evidence (Touratier and Goyet, 2011) indicates that all water masses in the Mediterranean Sea are already displaying decreases in pH of 0.05–0.14 units (compared to the global mean decrease of 0.1), and thus appears to be one of the regions that is most impacted by acidification (The Mermex Group et al., 2011).

The Mediterranean has proved enigmatic with respect to the characterisation of its diazotrophy and diazotrophic communities and to date there have been only a limited number of studies which have reported on this. Historically, indirect evidence from nutrient budgets (Bethoux and Copinmontegut, 1986) and stable isotope studies (Pantoja et al., 2002) indicate the potential for nitrogen fixation as an active process. Garcia et al. (2006) and Rees et al. (2006) provided some of the earliest direct measurements of N_2 fixation for the west and east basins respectively. The high rates reported by Rees et al. (2006) have not been repeated and it would seem that the upper limit is of the order of 17 $nmolL^{-1}d^{-1}$ as reported in the annual time-series of measurements made by Garcia et al. (2006) at the DYFAMED site in the northwestern basin. Krom et al. (2010) has argued that processes peculiar to the eastern basin preclude the budgetary requirement for nitrogen fixation and that P limitation in this region is too severe to allow diazotrophic activity. There is some degree of variability in the rates that have been reported. Low N_2 fixation rates of <0.15 nmol L^{-1} d^{-1} have been recorded in open waters across both basins (e.g. Ibello et al., 2010; Rahav et al., 2013; Ridame et al., 2011). During the BOUM cruise along a 2000 km transect from west to east, the mean rates observed in the western basin were higher than this at 0.63 ± 0.45 nmol L^{-1} d^{-1} (Bonnet et al., 2011), with maximum rates of 1.80 ± 0.19 nmol L^{-1} d^{-1} measured in the vicinity of the plume of the River Rhone. In a further time-series study at DYFAMED, Sandroni et al. (2007), recorded rates of between 2 and 7.5 nmol $L^{-1}d^{-1}$, with maximum rates recorded at 10 m depth during August. It would seem that the higher rates of N_2 fixation reported (Bonnet et al., 2011; Garcia et al., 2006; Sandroni et al., 2007) are associated with nutrient replete coastal environments. During a Saharan dust addition experiment in coastal waters of Corsica (Ridame et al., 2013) observed increases in rates of N_2 fixation up to ~ 1.3 ± ~1.0 nmol $L^{-1}d^{-1}$ from a background rate of ~0.2 nmol $L^{-1}d^{-1}$ following the addition of Saharan dust to surface waters. During the BOUM cruise the diazotroph community was dominated by picoplanktonic cyanobacteria affiliated to Group A, *Bradyrhizobium* and α proteobacteria (Bonnet et al., 2011). Additionally the filamentous cyanobacterium *Richelia intracellularis* was present at all stations sampled (Bonnet et al., 2011), and also in the coastal eastern basin (Zeev et al., 2008). In other coastal waters the presence of diazotrophs has been related to Archaea, Proteobacteria and Cyanobacteria (Man-Aharonovich et al., 2007; Le Moal and Biegala, 2009).

The current consensus is that diazotrophy occurs throughout the Mediterranean and similar to other variables which include oligotrophy and productivity (The Mermex Group et al., 2011) shows a decreasing trend from west to east. It would appear that coastal regions might support greater rates of N_2 fixation than the open waters of the Mediterranean Sea.

We report here on a mesocosm experiment performed in the Bay of Calvi (BC), Corsica, in the western basin of the Mediterranean during June and July 2012 during which the relationship between OA, N_2 fixation rate and diazotrophic community composition was investigated. This experiment, which is described in detail by Gazeau et al. (2017a), formed a contribution to the European project 'Mediterranean Sea Acidification under changing climate' (MedSeA; http://medsea-project.eu) which was launched in 2011 with the objective to assess uncertainties, risks and thresholds related to Mediterranean acidification at organismal, ecosystem and economical scales.

2. Methods

The Bay of Calvi is situated in the Ligurian Sea, on the northwest coast of Corsica in the Mediterranean Sea (Fig. 1). The bay is subject to little human disturbance and has been described as pristine (Richir and Gobert, 2014) with low river and sewage discharges supplying limited nutrients (Lepoint et al., 2004). The open sea provides the main external source of new nutrients, albeit seasonal, providing deep, nutrient rich waters during the N–NE winds which occur during winter and early spring and nutrient-poor surface waters during the more common SW winds (Skliris et al., 2001).