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Eutrophication and acidification: Do they induce changes in the dissolved organic matter dynamics in the coastal Mediterranean Sea?

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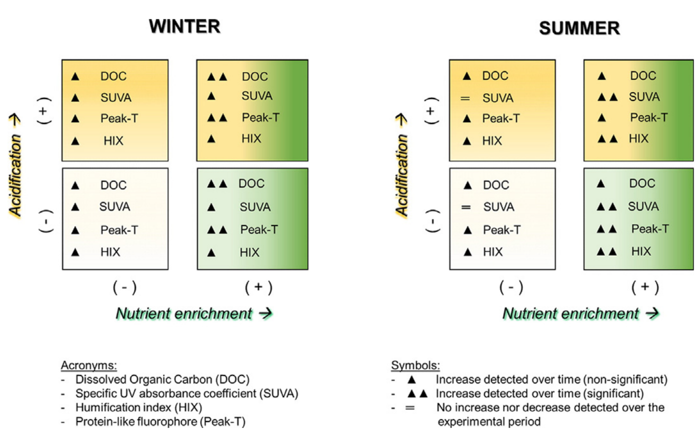
HIGHLIGHTS

- High pCO_2/nutrient levels effects in DOM dynamics were tested in a coastal system.
- Optical properties of DOM were used to track organic matter transformations.
- High pCO_2 did not significantly imbue transformations of DOM.
- Nutrient enrichment modified DOM dynamics in terms of quality and quantity.

GRAPHICAL ABSTRACT



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ABSTRACT

Two mesocosms experiments were conducted in winter 2010 and summer 2011 to examine how increased pCO_2 and/or nutrient concentrations potentially perturbate dissolved organic matter dynamics in natural microbial assemblages. The fluorescence signals of protein- and humic-like compounds were used as a proxy for labile and non-labile material, respectively, while the evolution of bacterial populations, chlorophyll *a* (Chl *a*) and dissolved organic carbon (DOC) concentrations were used as a proxy for biological activity. For both seasons, the presence of elevated pCO_2 did not cause any significant change in the DOC dynamics (p-value < 0.05). The conditions that showed the greatest changes in prokaryote abundances and Chl *a* content were those amended with nutrients, regardless of the change in pH. The temporal evolution of fluorophores and optical indices revealed that the degree of humification of the organic molecules and their molecular weight changed significantly in the nutrient-amended treatment. The generation of protein-like compounds was paired to increases in the prokaryote abundance, being higher in the nutrient-amended tanks than in the control. Different patterns in the magnitude and direction of the generation of humic-like molecules suggested that these changes depended on initial microbial populations and the availability of extra nutrient inputs. Based on our results, it is expected

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that in the future projected coastal scenarios the eutrophication processes will favor the transformations of labile and recalcitrant carbon regardless of changes in pCO_2.

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1. Introduction

As a result of human activities, atmospheric CO_2 levels have increased from approximately 280 ppm in pre-industrial times to 395 ppm in 2013 (Le Quéré et al., 2015 and references therein). A large portion of the atmospheric CO_2 is dissolved in the ocean and, thanks to the 'solubility pump', it is transported from the ocean's surface to its interior in form of dissolved inorganic carbon (Volk and Hoffert, 1985). In addition to this passive diffusion of CO_2 into the ocean, marine biota plays an active role in the uptake of carbon dioxide from the atmosphere in what is known as the 'biological pump' which refers to the processes that involve the biologically-mediated uptake and transport of carbon from the upper to the deep ocean (Volk and Hoffert, 1985; Passow and Carlson, 2012). Thus, marine ecosystems play an important role in regulating atmospheric CO_2 concentrations and, in this way, in moderating climate change. However, the physical, chemical, and biological mechanisms governing the fluxes between the different carbon compartments in the marine system are still poorly understood.

The diffusion of CO_2 into the ocean is determined by temperature and salinity that provide a dependent solubility coefficient (Henry's law, Henry, 1803). When a CO_2 molecule is finally taken up by the ocean, two main paths may follow: i) it may remain in a dissolved inorganic form, altering the marine carbonate chemistry equilibrium and leading to ocean acidification (Hönisch et al., 2012; Zeebe, 2012), or ii) may be captured by a photosynthetic marine organism, fixing it in the form of organic carbon. The pathways that this new biologically generated organic molecule may follow within the trophic chain are very diverse and vary from being incorporated into a larger organism (reaching higher trophic levels) to being excreted or respired as part of a variety of metabolic processes. The size of the excreted compounds varies widely, contributing to both the particulate organic matter (POM) and the dissolved organic matter (DOM) fractions. Regarding the DOM, this pool is mainly produced by phytoplankton exudation (Hopkinson et al., 2002; Romera-Castillo et al., 2011b; Sarmento et al., 2013), viral lysis (Brussaard, 2004; Motegi et al., 2009), the sloppy feeding carried out by protists and metazoans grazers and the POM solubilization by bacterial and archaeal hydrolases (Nagata et al., 2000; Sala and Güde, 2004). These mechanisms determine the quantity and the complexity of the molecules contained in the DOM, as well as their fate along the biogeochemical cycles.

The estimations of oceanic CO_2 assimilation by phytoplankton to generate cellular structures or its subsequent release of C as exudates (particulate and dissolved primary production, respectively) range between 3 and 4 Pmol C $year^{-1}$ (Berger, 1989; Antoine and Morel, 1996; Behrenfeld and Falkowski, 1997; Chavez et al., 2011). Research undertaken in the context of the US Joint Global Ocean Flux Study (Schlitzer et al., 2003) concluded that a fraction of this carbon is rapidly removed from surface waters and exported to the ocean's interior. In addition, Jiao et al. (2010) emphasized the role of oceanic in transforming POM and DOM into recalcitrant DOM, material susceptible of staying sequestered in the ocean for long periods of time. The processes that transform labile organic matter into refractory compounds are termed 'microbial carbon pump' (MCP, Jiao et al., 2010).

The chromophoric dissolved organic fraction (CDOM; Coble, 1996) of the DOM pool absorbs light at both ultraviolet (UV) and visible wavelengths. A sub-fraction of this CDOM, the fluorescent DOM (FDOM; (Coble, 2007, 1996), fluoresces when irradiated with UV light. Since 1990, (Coble et al., 1990) the characterization of marine DOM has been performed by applying fluorescence excitation–emission matrices (EEM). Although this technique does not permit the quantification of specific molecules, it has been extensively used to track the origin and transformations of DOM (Coble et al., 1990; Cory and McKnight, 2005; Nieto-Cid et al., 2005; Romera-Castillo et al., 2011a; Catalá et al., 2015) because it is relatively inexpensive, low-time consuming and provides valuable information about the quality of the DOM.

As it has been shown over the last years, ocean acidification affects marine organisms and ecosystems in several ways (Gattuso et al., 2015 and references therein). In addition, nitrogen (N) and phosphorous (P) pollution has increased over the past decades, primarily due to the utilization of active N and P for fertilizer use (Galloway et al., 2004). This utilization has enhanced the nutrient loads from land to coastal zones and may favor an increase of eutrophication episodes in the near future (Howarth and Marino, 2006). Since the beginning of the 20th century, eutrophication has been a persistent problem and a subject of different studies. Bio-assay experiments in lake and coastal systems were done to test the effect of eutrophication on phytoplankton dynamics in the seventies and eighties (Pomeroy et al., 1972; Carpenter and Capone, 1983). Since then, numerous studies have been addressed this issue in different aquatic systems (Statham, 2012 and references therein).

A convenient procedure to gain insight on the possible changes that ocean acidification and eutrophication may induce on marine systems is the deployment of mesocosms experiments (Kim et al., 2011; Teeling et al., 2012; Riebesell et al., 2007, 2013; Bunse et al., 2016). Three recent mesocosms studies (Yamada et al., 2013; Riebesell et al., 2013; Zark et al., 2015) have examined the effects of ocean acidification on DOM transformation processes. Yamada et al. (2013) did not find a significant effect of increased CO_2 concentration on the short-term decomposition of labile DOM in Sagami Bay (Japan), yet the study did not look at the possible changes in organic matter quality. The study conducted by Riebesell et al. (2013) in Svalbard (Norway) shed light on the pathways that the organic matter followed when the system was amended with nutrients and increased in pCO_2. They found that the combination of these two stressors triggered a synergistic effect inducing an increase in the dissolved organic carbon fraction. The study of Zark et al. (2015) tracked the transformations suffered by DOM molecules in a mesocosms study using Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) and they concluded that ocean acidification alone did not induce changes in the composition of the DOM pool in the Gullmar Fjord (Sweden).

We investigated the effects of increasing pCO_2 and its synergy with increasing nutrient availability on the dynamics of organic matter in a Mediterranean coastal area. We particularly examined the optically active fractions of the DOM, since they can be used as indicators of recalcitrant material and can provide useful information about DOM transformations. In addition, the study of these fractions is of remarkable interest in the Mediterranean waters where the CDOM to chlorophyll ratio is higher than the global average (Morel and Gentili, 2009; Claustre et al., 2002). We enclosed coastal water in mesocosms and performed two experimental studies in which we manipulated pCO_2 and nutrient concentrations. In order to assess the importance of the initial conditions in regulating the responses to reducing pH and increasing nutrients, one mesocosm experiment was performed in winter and the other in summer, displaying contrasting initial oceanographic and biological characteristics.

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