Volatile Organic Carbon Dynamics and Air-Sea Flux in the Coastal Ocean

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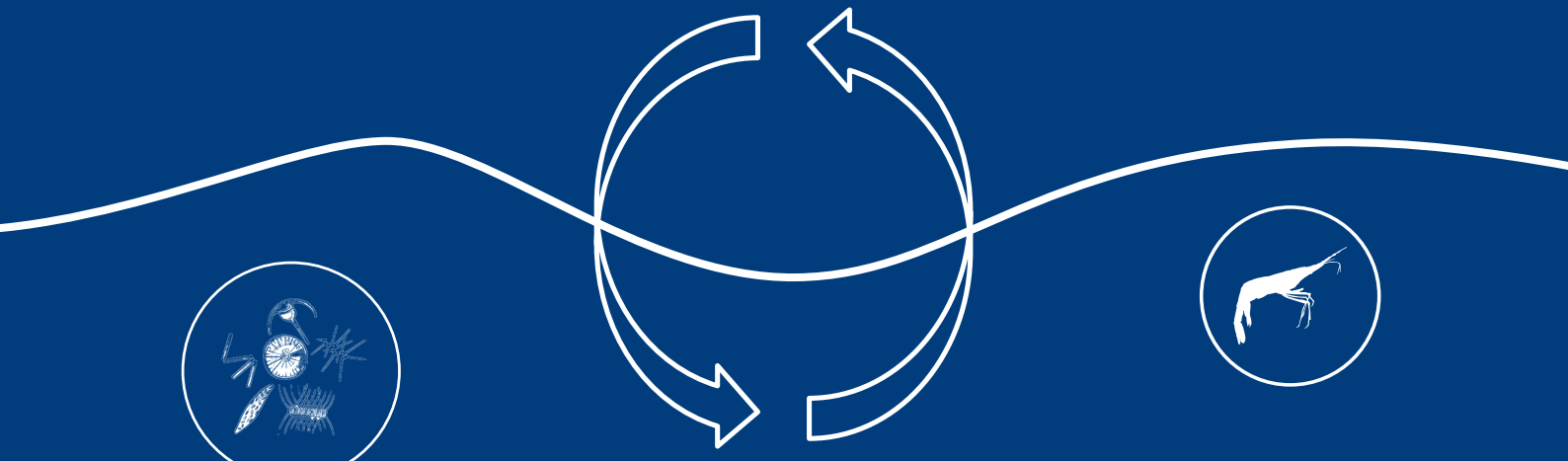

Universitat de les Illes Balears

Volatile Organic Carbon Dynamics and Air-Sea flux in the Coastal Ocean

Tesis Doctoral

Memoria presentada para optar al título de doctor por el Departamento de Biología.
Universidad de las Islas Baleares

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A mi familia, y

a mis amistades

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ABSTRACT

Because of its importance in biological processes, the regulation of the earth's climate and the human-induced changes to the global carbon cycle, assessing the global carbon budget is one of the most intensive activities in current biogeochemical research. In the oceanic domain the major pools of carbon (dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC)) are transferred with land and adjacent areas, through lateral exchanges, and gas and particle exchange with the atmosphere.

Although recognized on a single compound basis, the presence of total volatile organic carbon (VOC) and semivolatile organic carbon (SOC) and the exchanges thereof, between air and water have been ignored in current assessments of the global carbon budget in the ocean and atmosphere, largely due to methodological handicaps that precluded the estimation of bulk VOC and SOC in air and water. However, recent methodological developments have enabled the measurement of these bulk concentrations of VOC and SOC in water, termed exchangeable dissolved organic carbon (EDOC), and air, termed gaseous organic carbon (GOC), and the calculation of the air-water of flux of this pool of carbon

This method identified EDOC as an important fraction of total dissolved organic carbon in the ocean and established the air-sea exchange as a major pathway for organic carbon (OC), comparable in magnitude to that of CO_2. The research presented here aimed at evaluating the dynamics of VOC in coastal areas in relation to DOC and CO_2 exchange. This thesis identified cold environments in the Subarctic (Greenland) and the Southern Ocean (Antarctic Peninsula) as areas of active cycling of EDOC, with a prevalence of fluxes towards the ocean, consistent with lower temperatures that favor

the dissolution of gaseous phase OC in the water. Furthermore, several organisms and ecosystem compartments were identified as producers and consumers of

EDOC. Macroalgae, phytoplankton, Krill and sediments were found to contribute to the EDOC pool in the water, while the water column was a net sink for EDOC.

The work presented here reports daily, seasonal and interannual variability in the stocks of DOC, EDOC and GOC in the Mediterranean and points to an active role of EDOC and GOC in the biogeochemistry of the oceans, affected by, and with effects on, biological and physical-chemical processes, both in the water and the atmosphere. Moreover, the benthic compartment in the Mediterranean sites studied, EDOC represented around 10% of DOC flux and acted, generally, as a sink for EDOC driving the necessary deficit for the downward flux of gaseous phase OC.

Overall, this thesis highlights the ubiquitous nature of EDOC in the oceans, which represents, globally, 25-30 % of total (DOC+EDOC) in the water. This fraction is not small and has important implications for the biogeochemistry of the marine realm. Furthermore, the air-water flux of OC is not negligible and is likely to represent the major fraction of atmospherically derived carbon to the world's oceans, contributing to the redistribution of OC across large areas.

RESUMEN

Debido a su importancia en procesos biológicos, la regulación del clima en la tierra y los cambios provocados por el hombre en el ciclo global del carbono, evaluar el balance global de carbono es una de las actividades más intensas dentro del campo de las investigaciones biogeoquímicas. En el dominio oceánico los mayores acervos de carbono (carbono inorgánico disuelto (CID) y carbono orgánico disuelto (COD)) se transfieren con la tierra y áreas adyacentes mediante intercambios laterales, y con la atmósfera mediante intercambio gaseoso y de partículas.

Aunque reconocido para compuestos individuales, la presencia de carbono orgánico volátil (COV) y carbono orgánico semivolátil (COS) total y su intercambio con la atmósfera son ignorados en los balances actuales del ciclo del carbono en el océano y la atmósfera, debido, en gran parte, a problemas metodológicos que no permitan calcular la concentración total de COV y COS en aire y agua. Sin embargo, avances metodológicos recientes han permitido medir estas concentraciones totales en agua, denominadas carbono orgánico disuelto intercambiable (CODI), y en aire, denominadas carbono orgánico gaseoso (COG), y el cálculo del flujo aire-agua.

Esta metodología identificó al CODI como una fracción importante del carbono orgánico disuelto (COD) total y estableció el intercambio aire-agua como uno de los flujos de carbono orgánico (CO), comparable en magnitud con los flujos de CO_2. La investigación presentada aquí busca evaluar las dinámicas de COV en áreas costeras en relación al COD e intercambio de CO_2.

Esta tesis identificó ambientes fríos en el subártico (Groenlandia) y el océano sur (península Antártica) como áreas activas en el ciclado de CODI, con una prevalencia de flujos hacia el océano, consecuente con las bajas temperaturas que favorecen la

disolución de CO en fase gaseosa. Además, varios organismos y compartimentos ecosistémicos fueron identificados tanto como productores como consumidores de CODI. Macroalgas, fitoplancton, Krill y sedimentos contribuyen al CODI en el agua, mientras que la columna de agua es un sumidero de CODI.

Este trabajo demuestra la existencia de ciclos diarios, estacionales e interanuales de CODI y COG en el mediterráneo y apunta, una vez más, el papel activo de estos agentes en la biogeoquímica de los océanos, afectado por, y con efectos, tanto en procesos biológicos como en procesos físico-químicos, en el agua y en la atmósfera. Por otra parte, el compartimento bentónico del ambiente mediterráneo estudiado, CODI representa alrededor de un 10 % del flujo de COD y actúa generalmente, como sumidero de CODI, produciendo el déficit necesario para sustentar el flujo hacia el agua de CO en fase gaseosa.

En suma, esta tesis subraya el carácter ubicuo de CODI en los océanos, representando, globalmente, alrededor de un 25-30% del total (COD+CODI) en el agua. Esta fracción no es despreciable y tiene importantes implicaciones para la biogequímica en el océano. Además, el intercambio aire-agua de CO, comparable en magnitud al de CO_2, representa una fracción más grande del carbono derivado de la atmósfera en los océanos, contribuyendo a la redistribución de CO a lo largo de grandes distancias.

God is a gaseous vertebrate

Ernst Haeckel

(1834-1919)

GENRAL INTRODUCTION

The era for biogeochemistry

Compelling evidence for human influence on planetary processes (Vitousek et al. 1997) has lead to an increased attention on biogeochemistry as the central science to understand and manage human impacts on the global environment (Zak et al. 2006). Properly constraining the biogeochemical cycles of the elements and the impacts on these cycles associated with human activity is a central task for biogeochemists (Schlesinger 2004).

Indeed, the cycles of almost any element of economic importance in the periodic table have been significantly altered (Schlesinger 2004). Humans use about half of the available fresh water on earth (Postel et al. 1996), with future shortages rising as a major concern (Postel 2000), and mobilize large amounts of N and P, altering their natural availability, with long term impacts (Tilman et al. 2001). We have doubled the amount of S cycling through the planet with associated acid deposition events (Rodhe 1999), and dominate the mobilization of metallic elements in the biosphere (Nriagu 1989). To fully understand these biogeochemical cycles requires a particular focus on the linkages across boundaries (land, ocean, atmosphere), rather than considering these domains as closed systems where movements across these borders are of no consequence to ecosystems.

The carbon cycle

The carbon cycle is one of the most intensively studied biogeochemical processes, particularly for two reasons (1) it is central in ecosystem metabolism and is a

major by-product of industrial and agricultural activity (Allen et al. 2005); and, (2) carbon atoms are the building blocks that provide the scaffolding for the production of all biological structures, from simples molecules to whole organisms (Benner et al. 2004). Thus, the flow of energy (contained in chemical bonds), and materials (at rates determined by their biological and chemical stoichiometry), relies heavily on the cycling of this element. Extraction and burning of fossil fuels, together with cement production and changes in land use, with the concomitant release of CO_2 to the atmosphere, is the single most evident footprint of human activities on earth. The large amount of anthropogenic CO_2 added to the atmosphere, however, represents only 7% of the global flux of CO_2 exchanged to and from the atmospheric reservoir, but is enough to create worldwide concern over global climate change and a warmer planet (Schlesinger 2004). Hence, understanding the global carbon cycle is of paramount importance in our efforts to manage the global environment.

The carbon cycle is controlled by the transfer of carbon between land, atmosphere and the oceans. The latest assessments from the IPCC include inputs to the ocean from fresh water, in the form of particulate and dissolved organic matter, and inorganic carbon species, and exchanges with the atmosphere of CO_2, solely (Denman et al. 2007). The main oceanic pool of active carbon is the dissolved inorganic carbon (DIC) reservoir (McNichol et al. 1994), and influences carbon biogeochemistry through the solubility pump. However, dissolved organic matter (DOM), accounting for only 2% of the total carbon pool (Falkowski et al. 2000), remains the largest bioreactive pool of carbon, and influences carbon biogeochemistry through the biological pump (Hansell and Carlson 2002).

Trace gases in the environment

Despite extensive literature on the effects of marine and terrestrial originated gases on the chemistry of the atmosphere (Atkinson 2000), and the widespread occurrence of these trace gases both in the atmosphere and the ocean, whether from terrestrial (natural or anthropogenic) or marine origin (Williams 2004), the inventory of gas-phase organic carbon in the ocean and the atmosphere remains restricted to a discrete set of molecules (Goldstein and Galbally 2007). Furthermore, the flux of organic carbon associated with the air-sea exchange of organic trace gases, remains, to date, unresolved, and its importance not accounted for in current assessments of the global carbon budget of the planet (Denman et al. 2007)

Numerous terminology and classifications exist to categorize gas-phase organic compounds. The two most inclusive terms, only refer to their physical state: VOC (volatile organic compounds) and SOC (semivolatile organic compounds), depending on the individual physical thresholds that define their presence in the atmosphere in a gaseous form. Any carbon containing molecule other than CO and CO_2 and CH_4 that may exist in a gaseous form at typical temperature and pressure conditions can be included in these two categories. There is a growing number of VOC and SOC measured by current analytical techniques (Table 1) as individual compounds, but this definition makes VOC and SOC consist of virtually an infinite number of molecules with a total pool impossible to resolve through the addition of single compound concentrations (Goldstein and Galbally 2007). Considerable knowledge on the concentration, sources, cycling and functions in the ecosystem of individual compounds exist both on land and in marine environments, as exemplified by the numerous reviews, inventories and modeling exercises (Guenther et al. 1995; Guenther 1997;

Kesselmeier and Staudt 1999; Bunce et al. 1991; Fuentes et al. 2002; Helmig et al. 2009, cf. summary of processes in Fig. 1), and the wide array of sampling and detection methods available (table 1). However, previous attempts to measure the total concentration of VOC and SOC in the atmosphere (Roberts et al. 1998), did not allow the exchange between the atmosphere and the ocean to be resolved. When measured in water (MacKinnon 1979), VOC and SOC were estimated to comprise between 1% and 6% of TOC, and were considered negligible. As a consequence, no available estimates of the total pool of VOC and SOC in the ocean, the atmosphere, or the rates of exchange between them were available until recently.

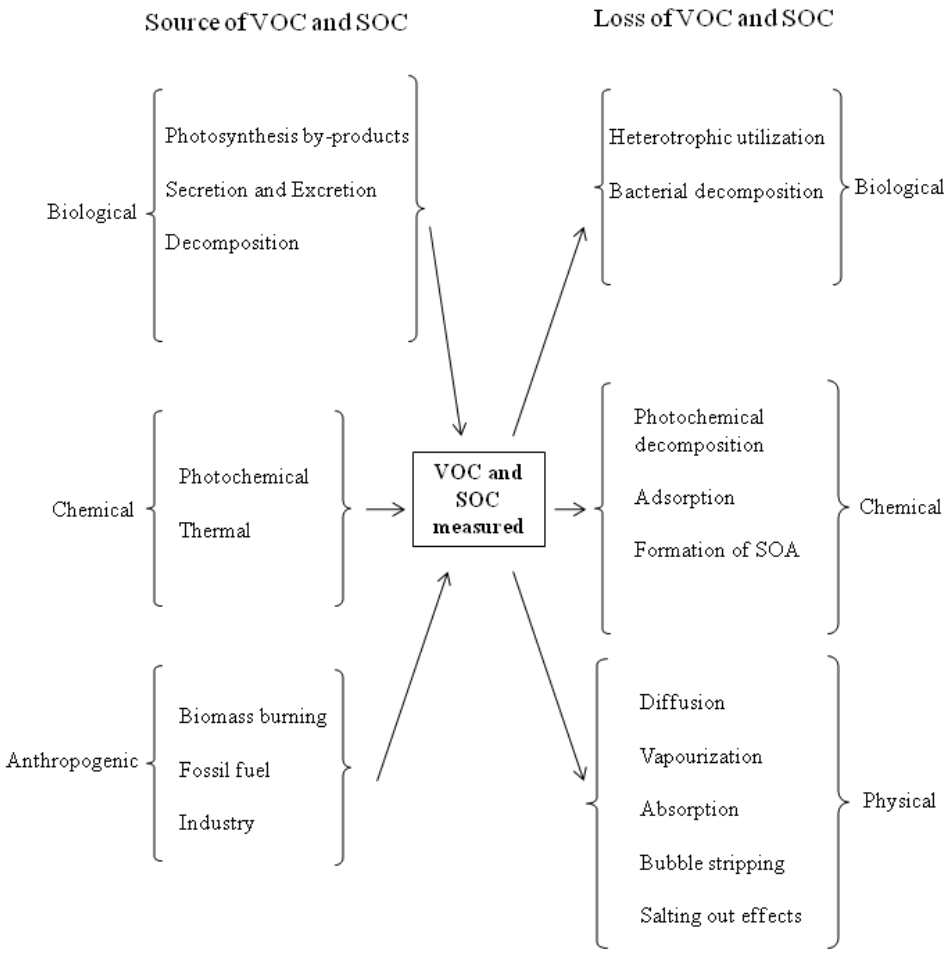

Figure 1. Diagram modified from Mckinnon (1979), showing biotic and abiotic processes involved in the production and destruction of volatile and semivolatile organic compounds (VOC and SOC). SOA stands for secondary organic aerosols.

However, a novel approach developed by Dachs et al. in 2005 was able to operationally measure the bulk, total pool of VOC and SOC dissolved in seawater, termed exchangeable dissolved organic carbon (EDOC) or the gaseous phase in the atmosphere, termed gaseous organic carbon (GOC). This methodology enabled, for the first time, the estimation of the pools of VOC and SOC in the ocean, the atmosphere, and the net flux between these two compartments, and the results revealed concentrations of EDOC (VOC and SOC) in seawater much larger than previously thought, supporting a net air-sea flux of organic carbon comparable in magnitude, or even higher, than the flux of CO_2 and aerosols combined (Dachs et al. 2005). This finding, lead to the realization that the flux of gaseous organic carbon could potentially be an important aspect of carbon biogeochemistry, that should no longer be neglected.

The coupled marine-atmosphere system

The global Oceans cover 71 % of the earth's surface and is in contact with 361 million km^2 of atmosphere (Siedler et al. 2001). Hence, these two closely coupled geophysical fluids exchange momentum, energy (heat) and materials (gas and particle exchange). In fact, major international consortia such as SOLAS (surface ocean lower atmosphere study, http://solas-int.org/) are entirely devoted to promote and coordinate research programs dedicated to the close relationship between the ocean and the atmosphere, encompassing a wide variety of disciplines from the earth and climate sciences. However, and despite the appropriateness of studying this processes under the umbrella of such programs, little attention is being paid to the establishment of a comprehensive global budget of total bulk VOC and SOC, and the exchanges thereof, between the ocean and the atmosphere.

Table 1. (Next page). Compilation of extraction and detection methods after Deemestere et al. 2007 and McKinnon 1979. *(Soil and sediment, sludge, biota, particulate matter). DAI: direct analysis injection. GC: gas chromatpgraphy ECD: electron capture detection. FID: flame ionization detection. HS: head space. HTCO: high temperature catalytic oxidation. MS: mass spectrometry. LLE:liquid-liquid extraction. SDE: steam distillation extraction. SDME: single drop microextraction. LPME: liquid phase microextraction. PLE: pressurized liquid extraction. DLLME: dispersive liquid-liquid microextraction. HPLC: high performance liquid chromatography. UV-vis: ultraviolet-visble light spectroscopy. SPE: solid phase extraction. SPME: solid phase microextraction. SBSE: stir bar sorptive extraction. INCAT: inside needle capillary adsorption trapping. GPE: gum phase extraction. OTT: open tubular trapping. FIMS: fiber introduction mass spectrometry. SPDE: solid phase dynamic extraction. AES: atomic emission spectroscopy. PTR: proton transfer reaction. APCI: atmospheric pressure chemical ionization. MMLE: microporous membrane liquid-liquid extraction. PME: polymeric membrane extraction. MASE: membrane assisted solvent extraction. MESI: membrane extraction with a sorbent interface. LGLME: liquid-gas-liquid microextraction.

extraction	Sample Matrix			Detection
	Air	Water	other*	
DAI		DAI	DAI	GC ECD FID
HS		static sampling dynamic sampling	static sampling	GC HTCO MS
CRYOGENIC	Trapping	Trapping Purge and Trapping		GC MS
SOLVENT	Impinger Denuder	LLE SDE SDME LPME DLLME HS-SDME HS-LPME	LLE PLE	GC MS HPLC UV-vis
IMMOBILIZED SORBENT	Adsorption tubes badges SPME FIMS cold-SPME GPE OTT In-tube SPME In-needle trapping	SPE SPME SBSE INCAT In-tube SPME OTT SPDE HS-SPME HS-SBSE SEP HS-SPDE Purge and sorbent trapping spray and sorbent trapping SLM	GPE OTT SPE SPME HS-SPME HS-SPME	GC MS AES PTR APCI
MEMBRANE	MIMS MESI	MMLE PME MASE MESI MIMS HF-LPME LGLME Purge and membrane extraction HS-MESI HS-MIMS	MIMS membrane based enrichment techniques	GC MS MIMS MS/MS PTR
OTHER			SPE RTIL	GC MS

Goal

The goal of this thesis is to measure the stocks and rates of exchange of VOC and SOC between the ocean and the atmosphere in a wide variety of coastal environments. Quantifying the total concentration of VOC and SOC in the ocean will allow us to assess the importance of this pool of organic carbon and compare it to that of DOC. Likewise, quantifying VOC and SOC in the atmosphere will allow the evaluation of the net exchange of gaseous-phase organic carbon between the ocean and the atmosphere, allowing comparisons with the exchange of CO_2. Determining the nature of the oceans as a source or sink, or with a dual capacity to absorb and emit VOC and SOC will allow us to better understand the exchange of organic carbon between the ocean and the atmosphere, which is not only relevant to improve our understanding of the global carbon cycle but also to better understand the influence of the ocean in tropospheric chemistry. In summary, the main objective of this thesis is to improve our understanding of the stocks, cycling, and air-water transfer of VOC and SOC and compare it to the pool of DOC and of CO_2 flux in a diversity of coastal ecosystems with the aim of evaluating its importance for the biogeochemical cycling of carbon in the coastal ocean. In particular we pose the questions: (1) is the pool of VOC and SOC an important component of TOC in coastal waters?; (2) are fluxes of VOC and SOC significant components of the carbon cycling of coastal ecosystems?; (3) are macrophytes, phytoplankton, and sediments sinks or sources of VOC and SOC in coastal ecosystems?

The following specific goals are pursued in each chapter:

Chapter 1. Evaluate the pool and exchange of VOC and SOC in air and water of cold marine coastal environments as areas of potentially large fluxes of OC towards the ocean, driven by the meteorological characteristics of subarctic regions, and identify possible sources and sinks in these ecosystems.

Chapter 2. Examine spatial and interannual variability in VOC and SOC pools and fluxes across the Antarctic Peninsula, a crucial area in the regulation of the earth's climate.

Chapter 3. Establish whether coastal organisms are sources or sinks of VOC and SOC. Examples of autotrophic and heterotrophic organisms have been selected. Furthermore, for primary producers, the size dependence in the cellular content and release of VOC and SOC has been established. The release of EDOC by Krill are presented as an annex to the journal article published in the pages of *Limnology and Oceanography* as the results ended up having no space in the context of the article, as they are preliminary. Nonetheless, the data are worthy on their own right.

Chapter 4. Assess the annual VOC and SOC budget of a coastal marine benthic habitat in the Mediterranean in relation to DOC production and the metabolism of the ecosystem.

Chapter 5. Asses the diel and annual cycles of DOC, VOC and SOC and examine the interannual variability and seasonality in VOC and SOC in the Mediterranean coast.

A not so brief note on methodology…putting the pieces together

The analysis of total organic carbon (TOC) in seawater, operationally discriminating between the particulate and dissolved pools by the selection of a filter of a determined pore size (typically 0.45 μm) has provided scientists with the necessary tools to evaluate the rates, stocks, movements and transformations of bulk organic carbon through the marine realm (Hansell and Carlson 2002). However, this analytical technique did not allow the characterization of individual compounds which is one of the directions in which enormous research progress is being made (Dafner and Wangersky 2002). On the other hand, in the opposite direction as DOC, VOC compounds have been routinely measured in the atmosphere, since the early 1950's. Still, today, VOC research mainly focuses on the characteristics and cycling of individual compounds, and new species are continuously added to the inventory (Kesselmeier and Staudt 1999). Yet, a comprehensive, quantitative estimate of total VOC and SOC in the oceans and atmosphere is still lacking (Goldstein and Galbally 2007).

The backbone of this thesis relies on a novel approach to measure total bulk VOC and SOC in the atmosphere, as well as the fraction dissolved in water developed by Dachs et al. (2005). This methodological breakthrough allows to operationally quantify, in a way similar to DOC, the total amount of organic carbon in gaseous form, whether dissolved in the water or in the atmosphere, readily transferable between air and water. Furthermore, it allows, for the first time, to estimate the total transfer of OC in gaseous form between the atmosphere and the ocean.

This methodology consists on the evolution of the volatile and semivolatile material in a sample and subsequent re-equilibration in pre-acidified pure water, where

it can be stored and measured with conventional high-temperature catalytic oxidation techniques (Dachs et al. 2005), as for DOC. Indeed, because the conventional approach to measuring dissolved organic carbon (DOC) includes bubbling of the acidified sample to remove inorganic carbon before analysis in a TOC analyzer (Spyres et al. 2000), conventional measurements of DOC represent, in fact, measurements of non-purgeable DOC. The purgeable or exchangeable fraction, not included in conventional DOC analyses, contains numerous VOC and SOC compounds and is operationally defined here as exchangeable dissolved organic carbon (EDOC). Likewise, VOC and SOC compounds present in the air are not measured directly but equilibrated in ultrapure water, and is referred to as GOC (gaseous OC), thus measuring GOC H'^{-1}, where H' is the Henry's Law constant. The advantage of resolving experimentally GOC H'^{-1} rather than measuring GOC in air and then calculating equilibrium concentrations in seawater using the Henry's Law constant is that H' is compound-specific, thereby requiring a complete inventory of the concentration of all VOC and SOC species present, as well as knowledge of their corresponding H', which are not yet possible.

Samples for EDOC determination are collected by bubbling 1-liter of sample with high-purity N_2 gas for 5–8 min, determined in laboratory experiments to suffice to reach equilibrium, to purge the volatile and semivolatile organic compounds, which are then trapped in 50 mL of ultrapure water free of carbon, preacidified with phosphoric acid to pH < 2. Subsamples from the ultrapure water trap containing the volatile compounds are then transferred to glass ampoules, precombusted at 450ºC for 4.5 h, and OC was analyzed in duplicate by high-temperature catalytic oxidation on a Shimadzu TOC analyzer, avoiding the sparge procedure to prevent EDOC losses (Spyres et al. 2000). Blanks were prepared in the field by bubbling the ultrapure water free of OC directly with high-purity N_2 gas for 5–8 min and analyzed for OC as

indicated previously. EDOC concentrations were calculated as the difference between carbon concentration in the samples and that in the blanks. Because samples are not purged, the blanks contain CO_2 present in the sample, which is substantial.

The efficiency of EDOC extraction by this procedure ranges depending on the degree of volatility of the compounds and was assessed to be 53 ± 28% and 80 ± 26% for acetone and toluene, respectively (Dachs et al. 2005). These recovery efficiencies, however, cannot be used to properly assess accuracy, nor correct to provide better estimates of bulk concentration, since these efficiencies are compound specific, and there are no reference materials for the complex pool of compounds included in our measurements, likely involving thousands of compounds. Duplicate EDOC, GOC and blank samples showed variability well above that for conventional DOC analyses, although duplicate analyses do not allow for statistical uncertainty to be properly estimated. We, therefore, pooled all the standardized duplicate estimates to examine the statistical distribution of the variability among duplicate pairs. This exercise, based on several hundred duplicated estimates, indicated that the median and mean standard deviation of the GOC and EDOC estimates is 5.2 μmol CL^{-1} and 6.3 μmol C L^{-1}.

The total concentration of OC in the atmosphere (GOC) was determined indirectly (operationally as GOC H'^{-1}) by equilibrating air and ultrapure water for 30 min, determined experimentally to suffice to equilibrate the concentration in water, by bubbling prefiltered air (QMA filter, Whatmann) through 50 mL of acidified (pH < 2 with H_3PO_4) ultrapure carbon-free water upwind from possible contamination sources. Subsamples of this ultrapure water were immediately transferred into precombusted glass ampoules (at 450ºC for 4.5 h), sealed, and analyzed in duplicate for OC as for EDOC. The OC concentration in the water represents the concentration of gas-phase

OC equilibrated in water and provides an operational estimate of GOC H'^{-1}, where H' is the dimensionless Henry's law constant.

There are two main reasons to measure GOC using this procedure instead of directly measuring GOC in air: (1) that the concentration of GOC in air is likely to be very low, difficult to resolve with available instruments, and (2) that our ultimate motivation to estimate GOC concentrations in air is to estimate the air–water flux, which depends on the differential between the GOC concentration in the air phase in equilibrium with the water (i.e., GOC H'^{-1}) and that in water (EDOC). Because the nature of the organic compounds contributing to the atmospheric GOC pool in air is unknown and since Henry's law constants regulating the partitioning between the air and water phase are compound specific, the approach developed by Dachs et al. (2005) allows calculations of air–water diffusive OC fluxes by experimentally resolving GOC H'^{-1} directly, thereby avoiding error in the flux estimations derived from assumptions on H'.

Diffusive air–water exchange was estimated using the wind speed dependence of the mass transfer velocity (k600) from instantaneous wind speeds (U10, m s^{-1}) following the equation $k600 = 0.24\ U10^2 + 0.061\ U10$ (Nightingale et al.2000). OC net diffusive fluxes (FOC) were estimated as the sum of gross volatilization (FOC,VOL= k' x EDOC) and absorption (FOC,AB= -k' x GOC H'^{-1}), where k' is the gas transfer velocity estimated from k600 values and Schmidt numbers assuming an average molecular weight (MW) of EDOC and GOC of 120 g mol^{-1} (Dachs et al. 2005). This arbitrary MW is chosen because it represents an intermediate value between volatile and semivolatile species, respectively (e.g., aromatics: benzene 78 g mol^{-1}, pyrene 202 g mol^{-1}; aliphatic: butane 58 g mol^{-1}, heptadecane 223 g mol^{-1}; polar: methanol 32 g mol^{-1}, diclorophenol 163 g mol^{-1}). Sensitivity analyses showed that estimates of k' are

relatively insensitive to MW values, as a variability of 50–200% over the value of 120 g mol^{-1} assumed here led to a variability in k' of 23.11% and 18.77% over the mean value, respectively, which is relatively small in comparison with the uncertainties associated with the wind speed dependence of k' and other factors affecting k' (Calleja et al. 2005).

The methodology used here presents several advantages, and enables to advance in the estimation of total organic carbon fluxes between the oceans and the atmosphere. However, there are also some caveats that deserve consideration. The concentration of EDOC and GOC should be considered minimum estimates, as equilibration times depend on the individual solubilities and vapour pressures of each compound and the temperature at which they were sampled. Indeed, the recovery efficiencies measured for Acetone and Toluene, indicate that a fraction of the EDOC pool will remain dissolved in the water, or will escape from the acidified water trap. Likewise some compounds may be removed by the bubbling procedure faster than others and may escape the trap water before the specified bubbling period is completed. Furthermore, field blanks add considerable uncertainty to the estimates and care in the cleaning and handling of the materials must be taken, rendering the work in certain conditions (e.g. small boats) quite challenging or impractical. Estimation of the total pool and fluxes would greatly benefit from further tests on optimizing equilibration times, better field blanks or radical new approaches in the measurement of total VOC and SOC as that recently described by Cueto et al. (2009), which would also enable the characterization of the isotopic signature of EDOC and GOC. Nevertheless, this new approach, if taken with caution, is likely to provide the scientific community with a new way to look at the relevance of VOC and SOC in the global environment.

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Unless you are the lead sled dog,

The view is pretty much the same

Inuit proverb

Chapter 1

Air–water exchange and vertical profiles of organic carbon in a subarctic fjord

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Abstract

In this article, we examine the air–sea exchange of exchangeable organic carbon (OC) as well as the internal pools and sources within a subarctic fjord. Air–water fluxes of OC ranged from an uptake of 22 ± 10 mmol C $m^{-2}d^{-1}$ in winter to a release of 2 ± 8 mmol C $m^{-2}d^{-1}$ in the fall, sizable compared to that of CO_2 (average uptake of 136 mmol C $m^{-2}d^{-1}$ for the fall and 2.6 ± 0.84 mmol C $m^{-2}d^{-1}$ in the winter). The water column profiles of exchangeable dissolved organic carbon (EDOC) followed closely those of dissolved organic carbon (DOC), and EDOC represented, on average, about one-third of DOC. The dynamic characteristic and active cycling of EDOC was evidenced by incubation experiments performed on each fjord compartment (sediment, water column, macroalgae) where sediments and macroalgae were found to be substantial sources and the water column acted as a strong sink of EDOC.

Introduction

Volatile organic compounds (VOCs) are important sources of carbon to the atmosphere (Guenther et al. 1995; Schauer et al. 2002) but have been only partially quantified. As the largest biome on Earth, covering 71% of the Earth's surface, oceans play a double role as a sink for terrestrially produced VOCs and semivolatile organic compounds (SOCs) (anthropogenic and of biological origin) (Jaward et al. 2004; Sinha et al. 2007) and as a source of volatile and semivolatile compounds released at different steps of the carbon flow in marine food webs (Steinke et al. 2002) or as a product of photochemical reactions (Laturnus 1996; Sinha et al. 2007).

Marine originated organic compounds are active in the atmosphere, affecting

tropospheric ozone concentrations (Monson and Holland 2001) and cloud formation (Vallina and Simó 2007), thereby contributing to regulate the Earth's climate (Williams 2004). Yet analyses of VOC fluxes focusing on individual compounds of particular interest (e.g., isoprene, DMS, halocarbons; Holmes et al. 2000; Gabric et al. 2001; Chuck et al. 2005) are dominant in the VOC literature (Williams et al. 2004; Sinha et al. 2007) but do not allow assessment of the quantitative importance of these compounds as a pool of carbon. In addition, SOCs also contribute to the overall flux of organic compounds in aquatic ecosystems (Dachs et al. 2005), especially when considering the fluxes of organic compounds from the atmosphere to the ocean (Jaward et al 2004).

As a result, the total flux of carbon associated with fluxes of VOC and SOC and their role in the carbon budget of marine ecosystems is still largely unresolved. The scarcity of data on the dynamics of dissolved volatile and semivolatile compounds within marine ecosystems is even greater (Laturnus et al. 1998), as research efforts have focused on a few individual compounds that are not necessarily representative of the total pool of volatile and semivolatile compounds in the oceans (Laturnus 1996; Sinha et al. 2007) and the sources and sinks of volatile and semivolatile compounds within marine ecosystems remain largely unresolved. A major reason for the present paucity of knowledge on marine VOC and SOC dynamics is methodological, as there were, until recently, no methods available to quantify total carbon concentrations associated with VOC and SOC in seawater. Previous attempts to quantify total organic carbon (TOC) in air (Roberts et al. 1998) did not suffice in themselves to estimate the exchange between air and water since it was measured directly in the atmosphere (see Methods for details).

Indeed, because the conventional approach to measuring dissolved organic carbon (DOC) includes bubbling of the acidified sample to remove inorganic carbon before analysis in a TOC analyzer (Spyres et al. 2000), the DOC measurements

represent, in fact, measurements of nonpurgeable DOC. The purgeable or exchangeable fraction, not included in conventional DOC analyses, was defined by Dachs et al. (2005) as exchangeable dissolved organic carbon (EDOC), as the approach to measuring this pool involves purging the water sample with a carrier gas free of OC. Whereas some companies manufacture purgeable OC modules for their OC analyzers (e.g., Shimadzu TOC-Vcsh), these instruments do not seem to yield adequate results, as recovery rates are very low in standard curves (47.78 6 2.37% recovery rate for Toluene on the Shimadzu TOC-Vcsh).

However, Dachs et al. (2005) developed a new approach based on the equilibration of EDOC between air and pure water by purging off EDOC from seawater samples and equilibrating in pure, carbon-free water to estimate total concentrations of EDOC in seawater. Similarly, equilibration of gas-phase organic carbon (GOC) in carbon-free water with that in air allows estimation of GOC concentrations in air indirectly by measuring their concentration equilibrated in water as GOC H'^{-1}, where H' is the dimensionless Henry's law constant.

This approach allows estimation of the direction and magnitude of air–sea OC exchange (Dachs et al. 2005). Using this approach, Dachs et al. (2005) demonstrated high concentrations of EDOC in the subtropical northeastern Atlantic, representing 30–40% of the DOC pool (Dachs et al. 2005), and identified significant atmospheric inputs of gas-phase organic matter to the subtropical Atlantic. These results confirm that EDOC can be a significant component of the C pool and fluxes in marine ecosystems of global relevance (Jurado et al. 2008). Dachs et al. (2005) provided data for the subtropical Atlantic. However, cold marine environments are areas of potentially large EDOC-GOC fluxes for a variety of reasons: (1) Henry's law constants (H') are low at low temperatures, displacing exchangeable OC to the water phase (Staudinger and

Roberts 2001); (2) arctic macroalgae have already been identified as a source of halogenated VOC and are abundant and widely distributed (Laturnus 2001); and (3) the presence of seasonal ice cover reduces the area for exchange, reducing fluxes between water and air during the wintertime.

Here we examine the concentration, air–sea exchange, and internal inputs and sinks of EDOC and GOC in a subarctic fjord ecosystem (Kobbefjord, southwestern Greenland), a rather contrasting environment to the subtropical open-ocean region where OC pools and fluxes were first assessed (Dachs et al. 2005). We assess the size of the EDOC pool relative to those of nonpurgeable dissolved and particulate OC pools. We then identify possible biological sources and sinks of EDOC and GOC and compare the magnitude of air–sea exchange of CO_2 and GOC for this ecosystem.

Methods

Kobbefjord is a 17-km-long, 0.8–2.0-km-wide deep sill fjord in Nuuk, capital of Greenland (Fig. 1). The average depth is 44 m, and the maximum depth is 120 m (Mikkelsen et al. 2008). The study took place between September 2007, before the onset of the ice-covered period, and May 2008, once the ice had already disappeared. During this time, the fjord was sampled in three different seasons; autumn, from 10 to 22 September 2007; winter, from 12 to 29 February 2008; and spring, from 13 to 27 May 2008. Sea ice is present seasonally in the fjord, with high interannual variation and typically covers the innermost part of the fjord (Mikkelsen et al. 2008). We gathered data on OC and CO_2 pools in the water column and the atmosphere. One station, located near the deepest area of the fjord, was repeatedly sampled in each sampling period to resolve changes in DOC, POC, and EDOC along the water column. At this station, five depths (1, 10, 20, 40, 80 m) were sampled using a 12-liter Niskin bottle, following casts

with a SeaBird 19 plus conductivity temperature depth (CTD) probe fitted with a fluorescence probe to profile properties in the water column. On most sampling occasions the fluorescence maximum was captured by the standard sampling depths, but on 11 September 2007, an additional sample was collected at 5 m depth to include the depth of the fluorescence maximum. Additional data from CTD casts performed through the year as part of a monitoring program were available to better characterize annual dynamics in the fjord.

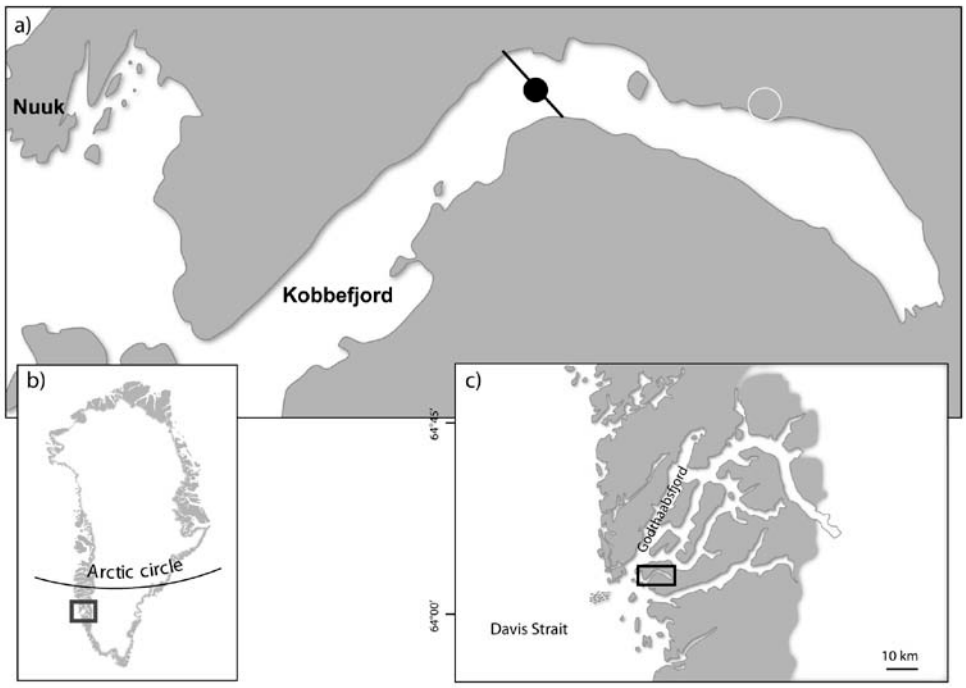

Figure 1. (A) Map of Kobbefjord showing the maximum sea ice extent (black line) and the location of the sampling station (closed circle). (B) The location of the Godthaabsfjord system in Greenland and (C) details of the Godthaabsfjord system.

In May 2008 we examined, using experimental incubations, the role of sediments, planktonic communities, and macroalgae, occupying the shallow margins of the fjord, in supporting EDOC fluxes. Six sediment cores (Plexiglas, 5.2-cm-diameter cylinders, containing 650 mL of overlying water) were taken at the fixed sampling station and incubated for 36 h at *in situ* temperature (2 ºC). Magnetic stirrers ensured

sufficiently well-mixed overlying water. Two of the cores were sampled every 12 h to measure EDOC concentration in the waters overlying the sediment and establish the net flux of EDOC from the rate of change in EDOC concentration over time. In an attempt to identify possible autochthonous sources and sinks of EDOC to the fjord and calculate the net EDOC production or consumption by the pelagic community, water from 20-m depth, just below the depth of the chlorophyll maximum, was collected in three replicate, acid-cleaned 1 liter glass bottles and incubated for 48 h at *in situ* light and temperature conditions. EDOC concentration was extracted following the method described below at the beginning and end of the incubation. *Laminaria longicruris* (de la Pylaie 1824) is among the dominant macroalgae in the fjord and was hence used to examine EDOC release by macroalgae. Macroalgae from the shallow parts of the fjord were collected and incubated for 72 h in gastight plastic bags filled with seawater at *in situ* light and temperature conditions. Each of the three replicate bags contained 55.20, 60.70, and 55.70 g wet weight of macroalgal tissue and 1.04 liters of seawater, purged with high-purity nitrogen to remove EDOC before use. EDOC was measured at the beginning and end of the incubation period. A conversion factor of 0.1767 was used to obtain dry weight (dry wt) from the wet-weight material (Wegeberg et al. 2006).

Water samples for determination of nonpurgeable DOC were filtered through precombusted fiberglass filters (Whatman GF/F) and frozen (-18 ºC) in precombusted and acid-washed 20-mL vials until analysis on a Shimadzu TOC-5000 analyzer (Shimadzu Corp.). Standards provided by Dennis A. Hansell and Wenhao Chen (University of Miami) of 2 μmol L^{-1} and 45 μmol L^{-1} DOC were used to assess the accuracy of the estimates. Samples for particulate organic carbon (POC) were measured on precombusted GF/F filters after filtration of 200–500 mL of seawater (volume depending on season). Filters were analyzed on an elemental analyzer (Robo-prep-CN,

Europa Scientific).

Exchangeable OC exists both in gaseous phase in the atmosphere and dissolved in seawater. The fraction of exchangeable OC dissolved in seawater is operationally quantified as EDOC (Dachs et al. 2005), and that present in the air is not measured directly but equilibrated in ultrapure water and is referred to as GOC (gaseous OC), thus measuring GOC H'^{-1}. Samples for EDOC and GOC were collected and analyzed following the procedures described in Dachs et al. (2005). Samples for EDOC determination were collected by bubbling 1 liter seawater samples with high-purity N_2 gas for 5–8 min, determined in laboratory experiments to suffice to reach equilibrium, to purge the volatile organic compounds, which were then trapped in 50 mL of ultrapure water free of carbon, preacidified with phosphoric acid to pH<2. Subsamples from the ultrapure water trap containing the volatile compounds were then transferred to glass ampoules, precombusted at 450 ºC for 4.5 h, and OC was analyzed in duplicate by high-temperature catalytic oxidation on a Shimadzu TOC-5000A or TOC-V-csh, avoiding bubbling to prevent EDOC losses. Blanks were prepared in the field by bubbling the ultrapure water free of carbon directly with high-purity N_2 gas for 5–8 min and analyzed for OC as indicated previously. EDOC concentrations were calculated as the difference between carbon concentration in the samples and that in the blanks.

The efficiency of EDOC extraction by this procedure ranges depending on the degree of volatility of the compounds and was assessed to be 53 ± 28% and 80 ± 26% for acetone and toluene, respectively (Dachs et al. 2005). These recovery efficiencies, however, cannot be used to properly assess accuracy, as there are no reference materials for the complex pool of compounds included in our measurements, likely involving hundreds of compounds. Duplicate EDOC and blank samples showed variability well above that for conventional DOC analyses, although duplicate analyses do not allow for

statistical uncertainty to be properly estimated. We, therefore, pooled all the standardized duplicate estimates to examine the statistical distribution of the variability among duplicate pairs. This exercise, based on 227 duplicated estimates, indicated that the median and mean standard deviation of the GOC and EDOC estimates is 5.2 µmol C $L^{-1}$and 6.3 µmol C L^{-1}.

The total concentration of OC in the atmosphere at the sampling station (GOC) was determined indirectly (operationally as GOC H'^{-1}) by equilibrating air and ultrapure water for 30 min, determined experimentally to suffice to equilibrate the concentration in water, by bubbling prefiltered air (QMA filter, Whatmann) through 50 mL of acidified (pH<2 with H_3PO_4) ultrapure carbon-free water upwind of the boat. Subsamples of this ultrapure water were immediately transferred into precombusted glass ampoules (at 450 ºC for 4.5 h), sealed, and analyzed in duplicate for OC as indicated previously. The TOC concentration in the water represents the concentration of gas-phase OC equilibrated in water and provides an operational estimate of GOC H'^{-1},where H' is the dimensionless Henry's law constant. There are two main reasons to measure GOC using this procedure instead of directly measuring GOC in air: (1) that the concentration of GOC in air is likely to be very low, difficult to resolve with available instruments, and (2) that the ultimate reason to estimate GOC concentrations in air is to estimate the air– water flux, which depends on the differential between the GOC concentration in the air phase in equilibrium with the water (i.e., GOC H'^{-1}) and that in water (EDOC). Because the nature of the organic compounds making the atmospheric GOC pool in air is unknown and since Henry's law constants regulating the partitioning between the air and water phase are compound specific, the approach developed by Dachs et al. (2005) allows calculations of air–water diffusive OC fluxes by experimentally resolving GOC H'^{-1} directly, thereby avoiding error in the flux

estimations derived from assumptions on H'.

Diffusive air–water exchange was estimated using the wind speed dependence of the mass transfer velocity (k600) from instantaneous wind speeds (U10, m s^{-1}) following the equation $k600 = 0.24\ U10^2 + 0.061\ U10$ (Nightingale et al. 2000). OC net diffusive fluxes (FOC) were estimated as the sum of gross volatilization (FOC,VOL = *k*' x EDOC) and absorption (FOC,AB =-*k*' x GOC H'^{-1}), where *k*' is the gas transfer velocity estimated from *k*600 values and Schmidt numbers assuming an average molecular weight (MW) of GOC of 120 g mol^{-1} (Dachs et al. 2005). This arbitrary MW is chosen because it represents an intermediate value between volatile and semivolatile species, respectively (e.g., aromatics: benzene 78 g mol^{-1}, pyrene 202 g mol^{-1}; aliphatic: butane 58 g mol^{-1}, heptadecane 223 g mol^{-1}; polar: methanol 32 g mol^{-1}, diclorophenol 163 g mol^{-1}). Sensitivity analyses showed that estimates of *k*' are relatively insensitive to MW values, as a variability of 50–200% over the value of 120 g mol^{-1} assumed here led to a variability in *k*' of 23.11% and 18.77% over the mean value, respectively, which is relatively small in comparison with the uncertainties associated with the wind speed dependence of *k*' and other factors affecting *k*' (Calleja et al. 2009). Data on wind velocity and air temperature were obtained from local weather stations (Jensen and Rasch 2008, 2009), and appropriate solubility corrections were applied to estimate in situ air–water OC fluxes. By convention, we use negative fluxes to denote inputs from the atmosphere to the ocean and positive ones to denote volatilization to the atmosphere.

The partial pressure of CO_2 in the water (pCO_2) was measured using a nondispersive infrared gas analyzer (Environmental Gas Monitor (EGM)-4, manufactured by pp systems) that measures pCO_2 with a precision of $\pm 10^{-6}$. For atmospheric measurements, air is passed through a desiccation column (anhydrous

calcium sulfate, Drierite) removing water vapor from the air in order to avoid interferences in CO_2 measurements. For pCO_2 I water, below-surface water was collected and passed through a gas exchange column (Mini-Module Membrane Contactor) and pCO_2 measured as described previously. Measurements correspond, therefore, to those in dry air, as measured, and no efforts were made to correct this for the atmospheric moisture as proposed in Calleja et al. (2005). Details of this methodology have been described elsewhere (Calleja et al. 2005; Silva et al. 2008). CO_2 exchange between air and water was estimated using short-term wind speed dependence (Wanninkhof and McGillis 1999).

Results

The fjord started freezing on 05 January 2008, and the maximum ice extension was reached on 01 February 2008. The inner part was ice covered for almost 5 months (until 23 May 2008; Fig. 2), although some ice cover was retained at the land end of the fjord over much of the study. The water column at Kobbefjord remained well mixed, and plankton biomass was low throughout the ice-covered period. Following the ice melt in the spring, freshwater inputs progressively reduced salinity in the water column, concurrent with rising temperatures, and a fluorescence peak corresponding to a spring phytoplankton bloom developed soon after the ice melt (Fig. 2).

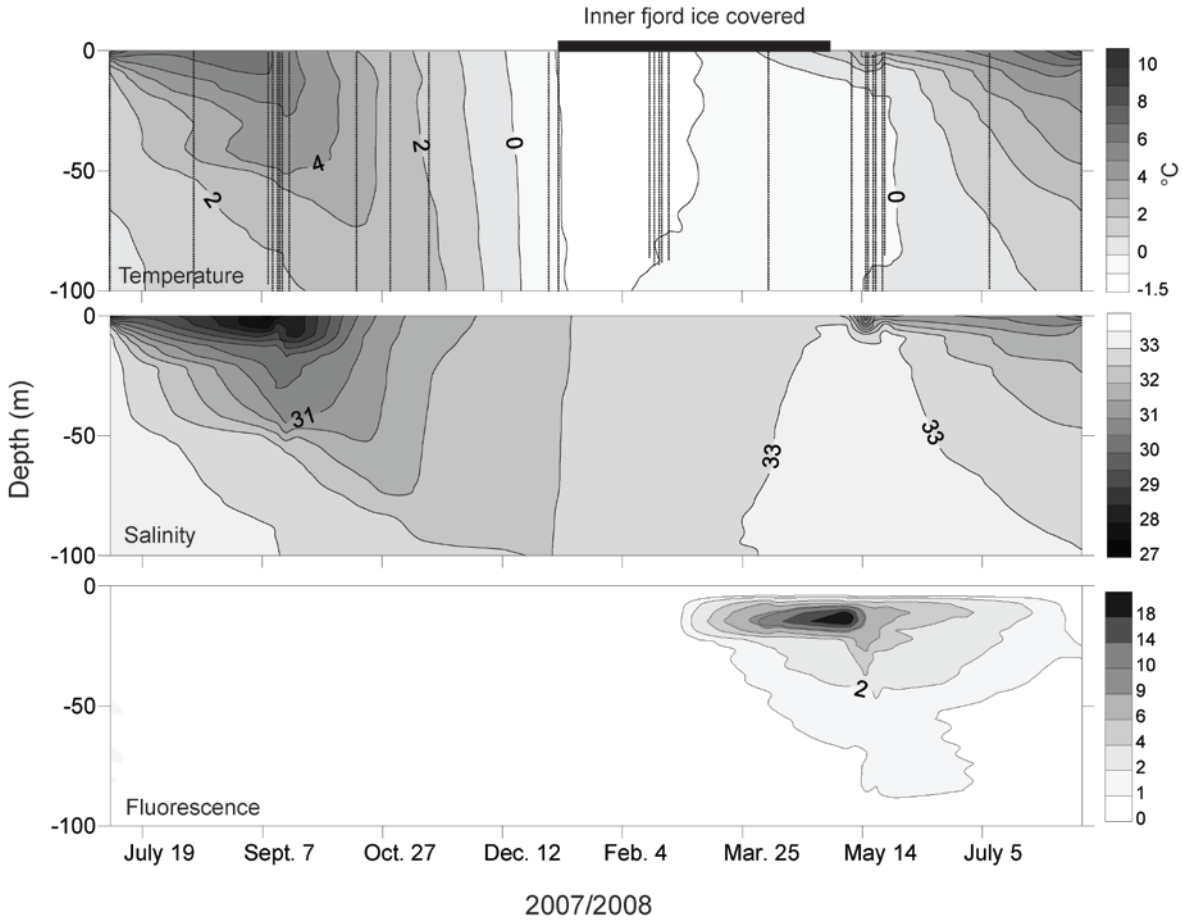

Fig. 2. (A) Temperature, (B) salinity, and (C) fluorescence distribution at the sampling station in Kobbefjord. Data obtained from regular CTD casts shown as vertical dotted lines on panel A. The duration of sea ice cover in the inner fjord is indicated by the black bar above panel A.

DOC concentrations varied between a minimum of 74 µmol C L-1 and a maximum of 210 µmol C L-1. EDOC at the permanent sampling station ranged from below the detection limit to a maximum of 133 µmol C L-1 and was particularly high in February, when the fjord was partly ice covered (Fig. 3; Table 1). Integrated EDOC pools were highest in February 2008 and lowest in May 2009 (Table 1). EDOC and DOC concentrations followed parallel trends with depth. EDOC concentrations were, however, much lower than those of DOC (Fig. 3; Table 1), typically representing about a third of total OC (TOC = DOC + EDOC + POC) but reaching values close to 50% in some instances (Table 1). POC represented a small fraction (on average 0.53 ± 0.03 mol

m^{-2} = 3.68% of TOC), but spring values reached twice the level of fall and winter values (Table 1). GOC concentration ranged from 13 µmol C L^{-1} to 79 µmol C L^{-1} and averaged 42 ± 5 mmol C L^{-1} along the study.

Table 1. Inventory of air–sea fluxes of OC and CO_2 (positive values denote fluxes to the atmosphere, and negative values represent an inward flux into the water) and OC pools—EDOC, DOC, POC, and proportion of TOC accounted for by EDOC—in the water column of Kobbefjord (southwestern Greenland) at different dates along the study. When error estimates are not reported, only two values were estimated, and no standard error (SE) could be calculated.

Season	**Date**	**air-water exchange (mmol m^{-2} d^{-1})**		**water column OC pools (mol m^{-2})**			
		CO_2	**OC**	**EDOC**	**DOC**	**POC**	**% EDOC**
fall	110907		15.73	4.05	7.55	0.36	33.9
	140907	-32.64	-1.37				
	180907	-240.5	-9.16	3.25	7.82	0.35	28.4
	Mean ± S.E	**-136.57**	**1.73 ± 7.35**	**3.65**	**7.69**	**0.36**	**31.15**
winter	150208		-37.66				
	170208	-2.81	-42.33	11.09	12.63	0.34	46.1
	190208	-1.07	1.79				
	200208	-3.98	-0.73	5.25	18.91	0.36	21.4
	230208		-30.82				
	Mean ± S.E	**-2,62 ± 0,84**	**-21.95 ± 10.47**	**8.17**	**15.77**	**0.35**	**33.75**
spring	150508		-4.54	2.40	10.82	0.81	17.1
	160508	-1.63	-2.22				
	180508	-1.88	-3.18	1.81	9.68	0.78	14.7
	190508	-4.61	0.23				
	220508		-2.41	1.03	13.74	0.72	6.7
	230508		-2.93				
	Mean ± S.E	**-2.50 ± 0.70**	**-2.50 ± 0.70**	**1.75 ± 0.4**	**11.41 ± 1.2**	**0.77 ± 0.03**	**24.05 + 5.39**

GOC concentrations were closely correlated with those of EDOC (R^2 = 0.73; Fig. 4), as expected from the dynamic equilibrium between these fractions, but GOC concentrations tended to exceed those of surface EDOC with GOC values exceeding EDOC concentrations in 10 out of the 14 paired samples collected by, on average, 45 ± 20%. Seven out of the 14 paired samples were obtained at the permanent station, and the remainder was obtained from the synoptic survey throughout the whole fjord.

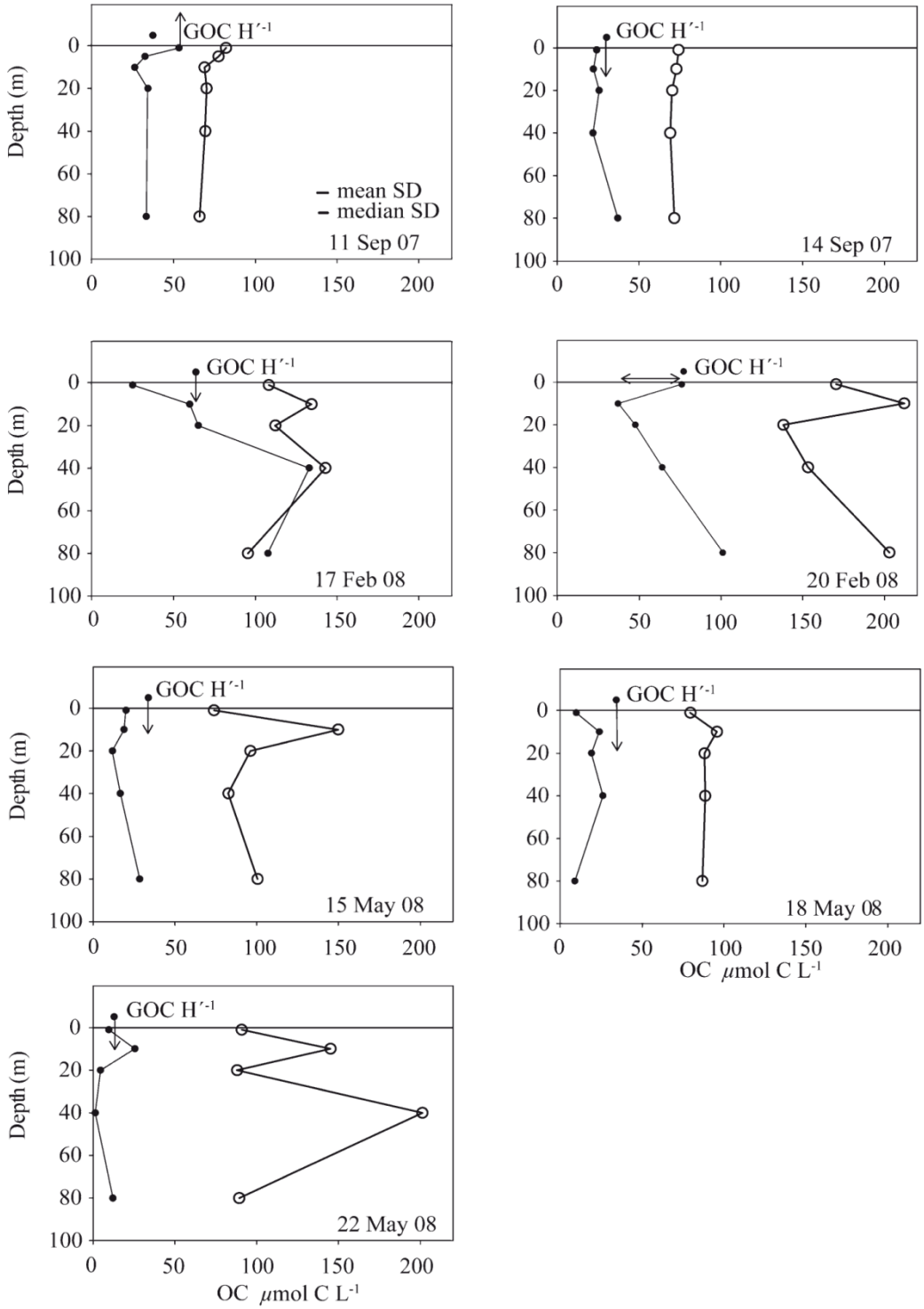

Fig. 3. Vertical profile of EDOC (closed circles), DOC (open circles), and GOC (large single dot) in µmol C L^{-1} at the sampling station in Kobbefjord, Greenland. Arrows represent the direction of exchangeable OC flow between the atmosphere and the water (horizontal arrows indicate no significant net flux). Mean (6.3 µmol C L^{-1}) and median 5.2 µmol C L^{-1} standard deviations of replicate analysis are shown as scaled bars of the X axis in the first panel for reference.

The tendency for the equilibrium concentrations of atmospheric GOC to exceed OC concentrations in fjord surface waters (EDOC) indicates a prevalence of a net diffusive flux of GOC from the atmosphere to the fjord waters (Table 1). The diffusive flux of GOC from the atmosphere to the fjord waters did not differ significantly from zero in the fall, as both influx and efflux were obtained in measurements conducted in the fall campaign (Table 1). A strong flux from the atmosphere was detected in. February. In May 2008 the transport of GOC was also directed from the atmosphere to the fjord water but was much smaller than that in winter (Table 1). CO_2 fluxes were always from the atmosphere into the water and were highly variable, spanning two orders of magnitude across measurements (Table 1).

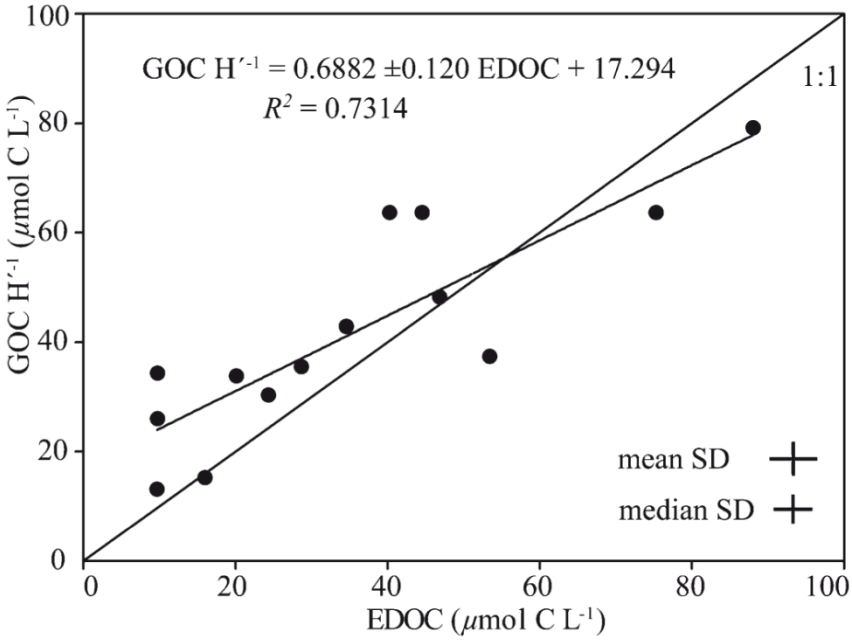

Fig. 4. The relationship between the equilibrium concentration of OC in the gaseous phase in the atmosphere (GOC) and the EDOC in surface water. The lines show the 1:1 line and the fitted least squares regression equation, shown in the figure ($p<0.0001$). Mean (6.3 μmol CL^{-1}), and median 5.2 μmol C L^{-1} standard deviations of replicate analysis are shown as scaled bars of the X and Y axes for reference.

Experiments to elucidate the role of various components of the fjord ecosystem as sinks or sources of EDOC were conducted in May 2008. Incubation of intact sediments led to an increase in EDOC concentration in the water column over time, indicative of a role of sediments as sources of EDOC to the water column at a calculated average rate of 4.8 mmol C $m^{-2} d^{-1}$. The planktonic community appeared to act as a strong sink of EDOC, with a net decline in EDOC of 3.32 ± 0.64 μmol C L^{-1} d^{-1} derived from incubations of water collected at 20 m in May 2008. Incubations of macroalgal blades also showed these to release EDOC at an average rate of 0.44 ± 0.19 μmol C g dry $wt^{-1} d^{-1}$.

Discussion

Our measurements indicate that EDOC is an important component of total OC in the water column of Kobbefjord, with EDOC representing, on average, 24 ± 5% of TOC concentrations and 37 ± 10% that of total (purgeable and nonpurgeable) DOC concentrations. This is comparable to the contribution of 30–40% of EDOC to DOC reported by Dachs et al. (2005) for the subtropical northeastern Atlantic and provides additional evidence that neglecting exchangeable OC leads to a significant underestimation of total DOC concentration in ecosystem carbon budgets, as the DOC concentrations reported in the literature refer to the nonpurgeable fraction. However, EDOC values were highly variable not only among seasons but also between dates in the same season.

The integrated EDOC pool was highest in the ice covered period (February), when the ice cover prevented equilibration with the atmosphere over much of the fjord, compared to the open-water periods of the study (Fig. 4) and presumably also because of the significantly lower H' values at lower temperatures, thus favoring the retention of

exchangeable OC in seawater. However, GOC concentrations (measured here as GOC H'^{-1}) in the atmosphere were also large in February, so that, despite the high EDOC concentration in seawater, the flux would still be from the atmosphere to the water column in the areas free of ice cover (Table 1), consistent with reports of enhanced deposition of semivolatile organic compounds at low temperatures due to low H' values (Satudinger and Roberts 2001).

Elevated winter EDOC concentrations in seawater could be accounted for by low respiration rates and low temperature, together with low light and UV levels, thereby minimizing potential losses of EDOC. In addition, ice communities have been reported to release DOC (Thomas et al. 1995) and could also act as sources of EDOC to the fjord, although this possibility was not evaluated here. In any case, the large EDOC concentrations in winter are indicative of a prevalence of EDOC sources over losses and low temperatures displacing the air–water equilibrium towards the water.

Air–sea OC fluxes were generally lower than those reported by Dachs et al. (2005) for the subtropical Atlantic Ocean, except in winter, when potentially large fluxes over the ice-free regions of the fjord were comparable in magnitude to those reported by Dachs et al. (2005). The flux of OC was significant compared to that of CO_2, with a larger flux of CO_2 into the fjord compared to that of exchangeable OC, except in May, when both fluxes were comparable in magnitude (Table 1). Whereas CO_2 samples were relatively uniform within sampling periods, OC air–water fluxes changed greatly within sampling periods, including changes in the direction of the OC flux (Table 1).

The vertical profiles of EDOC presented (Fig. 3) suggest the dynamic cycling of EDOC in Kobbefjord, with active consumption and production processes taking place through the water column, as also evidenced by the incubation experiments performed.

Kobbefjord acted as a sink for GOC, with an air–water flux of about 3.12 ± 1.6 mol C m^{-2} yr^{-1}. This estimate should be considered an approximation, as it was calculated from three sampling campaigns, and better knowledge of year-round air–sea exchange would be necessary to improve the accuracy and precision of this estimate.

The flux of GOC into the water column must be supported by a net consumption of EDOC in the ecosystem, maintaining the necessary EDOC deficit in the water column. Indeed, the experiments conducted in May point to a large consumption of EDOC in the water column, at a rate of 3.32 ± 0.64 mmol C $m^{-3}d^{-1}$ at 20-m depth. This is consistent with the vertical profiles observed in May, which show a local minimum at this depth (Fig. 4). On the other hand, the maximum in EDOC concentration at 10-m depth (Fig. 4), the depth of maximum net primary production, suggests that the planktonic community acts as a source of EDOC to the ecosystem. In addition, four of the seven profiles showed an increase in EDOC concentration toward the deeper waters of the fjord (Fig. 4), suggesting the presence of a source of EDOC at depth. This suggestion was supported by the experiments conducted in May, which revealed the sediments to be a significant source of EDOC to the ecosystem, with an average input of 4.8 mmol C $m^{-2}d^{-1}$ to the overlying water column, about twice as high as that derived from the atmosphere in this period (2.5 ± 0.7 mmol C $m^{-2}d^{-1}$; Table 1).

The benthic EDOC flux is likely supported by the release of volatile and semivolatile compounds during the decomposition of planktonic-derived POC, which would require, at sedimentation rates of 39 ± 3 mmol C $m^{-2}d^{-1}$ estimated using sediment traps during this campaign, the release as EDOC of about 12% of the POC reaching the sediment. Finally, our experiments also identify the macroalgae in the shallow areas of the fjord as significant sources of EDOC to the ecosystem, consistent with previous reports—focused on individual compounds (Laturnus 1996; Laturnus et al. 1998)—that

Arctic macroalgae are significant sources of VOCs.

Although our results do not suffice to attempt a budget of EDOC for the ecosystem, which would require accounting for lateral exchanges as well, they do identify the atmosphere and benthic communities (bare sediments and macroalgal beds) as sources of EDOC. Our results also show that EDOC minima in the water column correspond to consumption of EDOC in the water column and suggest that deep chlorophyll maxima, associated with EDOC peaks, may be sources of EDOC to the water column.

In summary, the data presented here support the importance of VOCs and SOCs for the carbon budget of the subarctic fjord investigated. The results show that exchangeable DOC represents a large and dynamic carbon pool within the subarctic fjord studied, accounting for about one-quarter of total OC in the water column. The atmosphere and benthic compartments acted as sources of EDOC to the water column, which could be consumed in the water column. The mean GOC flux from the atmosphere to the water column of 9 ± 4 mmol C $m^{-2}d^{-1}$ at Kobbefjord is well below the rates of 25 to 31 mmol C $m^{-2}d^{-1}$ reported by Dachs et al. (2005) for the subtropical northeastern Atlantic. However, GOC and EDOC pools and fluxes at Kobbefjord were sufficiently large as to be significant components of the carbon budget of this ecosystem. Our results for a subarctic fjord, therefore, add to those of Dachs et al. (2005) to show that VOCs and SOCs can be an important and dynamic component of the marine carbon pool. Yet this pool is grossly understudied and neglected from most accounts of carbon budgets in marine ecosystems. There is, therefore, a pressing need to characterize exchangeable OC concentration and dynamics as a step toward improving our understanding of carbon cycling in marine ecosystems.

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We must always remember

With gratitude and admiration

The first sailors that steered their vessels

Through storms and mists,

And increased our knowledge

Of the lands of the ice in the South

Roald Amundsen

1872-1928

Chapter 2

Ocean-Atmosphere exchange of organic carbon and CO_2 in the Antarctic Peninsula. Physical and biological controls

Sergio Ruiz-Halpern, María Ll. Calleja, Jordi Dachs, Damiá Gomis and Carlos M. Duarte

Manuscript, 2011

Abstract

Exchangeable organic carbon (OC) dynamics and CO_2 fluxes in the Antarctic Peninsula during austral summer were highly variable. By stations, the region was a weak source of CO_2 to the atmosphere, however, continuous records of CO_2 revealed this area as a weak sink. OC fluxes were also in both directions but generally towards the ocean and were much higher than CO_2 fluxes, sometimes by a factor of 10. Surface exchangeable dissolved organic carbon (EDOC) had a 43 ± 2.96 μmol C L^{-1}overall mean and gaseous organic carbon (GOC) 46 ± 3 μmol C L^{-1}, which represents around 66% of surface dissolved organic carbon (DOC) in Antarctic waters. There was a significant linear relationship between Partial pressure of CO_2 in the water and Chl *a* and krill density. However, such relationships were not found for EDOC. Depth profiles of EDOC were also quite variable and followed Chl *a* profiles, but only in some instances, while diel cycles of EDOC revealed two distinct peaks around midday and midnight, concurrent with UV maxima and krill night migration patterns. No evident diel pattern for GOC was found. The pool of exchangeable OC reveals itself as an important compartment of the carbon budget in the Antarctic Peninsula and adds to previous studies highlighting its importance in the redistribution of carbon in marine environments.

Introduction

The ocean and the atmosphere exchange momentum, heat, gas and materials across 361 million Km^2, with these interactions playing a major role in the functioning

of the Earth's System (Siedler et al. 2001). Gas exchanges play a key role in climate regulation, as the ocean has already absorbed a large fraction of anthropogenically produced CO_2 (Sabine et al. 2004), the major green house gas (ghg) contributing to global warming. However, the ocean acts as a source of other ghg's, such as methane (Judd et al. 2002) and dimethyl sulfide (DMS) (Charlson et al. 1987, Ayers and Gillett 2000). Likewise, there is a wide variety of volatile and semivolatile organic compounds (VOC and SOC) for which a comprehensive inventory is lacking as yet.

Goldstein and Galbally (2007) predicted that over 1 million C10 compounds are likely to exist in the atmosphere, making the pool of exchangeable carbon between the ocean and the atmosphere, virtually infinite and impossible to measure on a single compound basis. Hence, the few relevant volatile compounds routinely measured in marine organisms (Laturnus 2001; Sinha et al. 2007), which account for a small fraction of the VOC and SOC exchanged with the atmosphere, do not allow a quantitative estimation of the air-water exchange of organic carbon. There are numerous examples in the available literature of studies that demonstrate the production of single compounds or, at best, a modest set of individual VOC and SOC by marine organisms, from macrolagae to phytoplankton (Laturnus et al. 2000; Bravo-Linares et al. 2007). These studies demonstrate that the production of exchangeable organic carbon is ubiquitous in the ocean (Giese et al. 1999). However, quantifying the total amount of volatile organic carbon exchanged between the oceans and the atmosphere remains challenging.

However, VOC and SOC compounds can be collectively measured as exchangeable dissolved organic carbon (EDOC), if measured in the water and gaseous organic carbon (GOC), if measured in the atmosphere (Dachs et al. 2005; Ruiz-Halpern et al. 2010), thereby adopting an approach comparable to that of total organic carbon

(TOC) or dissolved organic carbon (DOC) to operationalize the quantification of these compounds beyond the limitations associated to approaches based on individual compounds (Dachs et al. 2005). EDOC and GOC are exchanged dynamically across the air-sea interface, a process that has been largely overlooked in current assessments of the carbon budget of the oceans (Denman et al. 2007). Yet, available studies identify the air-water exchange of organic carbon as an important component of the carbon budget of the subtropical NE Atlantic (Dachs et al. 2005) and subarctic fjords, where these fluxes have been quantified (Ruiz-Halpern et al. 2010). Indeed, as predicted by Jurado et al. (2008) these fluxes are comparable in order of magnitude to the fluxes of CO_2, and organic aerosols combined.

Polar ecosystems are areas of intense biological activity where the production and consumption dynamics of exchangeable organic carbon are likely to be of regional, or even, global relevance. Indeed, Ruiz-Halpern et al. (2010) identified cold marine environments as areas supporting, potentially, large air-sea OC fluxes for a variety of reasons: (1) Henry's law constants (H') are low at low temperatures, displacing exchangeable OC to the water phase (Staudinger and Roberts 2001); (2) polar macroalgae (Laturnus 2001), and phytoplankton (Sinha et al. 2007) have already been identified as an important source of a range of VOCs, including halogenated VOCs, methanol, acetone, acetaldehyde, DMS and isoprene, and are abundant and widely distributed (Laturnus 2001, Sinha et al. 2007); and (3) the presence of seasonal ice cover reduces the area for exchange, reducing fluxes between water and air during the wintertime leading to a potential large release during summer ice melt (Ruiz-Halpern et al. 2010).

Additionally, UV radiation, particularly high in the Antarctic spring and summer (Madronich et al. 1998), may affect the stocks of exchangeable organic carbon in the

water column by influencing phytoplankton cell death rates (lysis and subsequent release of OC to the environment, Llabrés and Agustí 2010), as well as through photochemical degradation of organic molecules, both in the water and the atmosphere (Zepp et al. 1998).

The Southern Ocean is particularly important in the regulation of the earth's climate, as the Antarctic circumpolar current connects all oceans, and is a significant sink for CO_2 (Sabine et al. 2004; Gruber et al. 2009). Understanding the carbon budget of the Southern Ocean, is, therefore, of particular interest. Unfortunately, whereas the air-sea exchange of CO_2 has been evaluated extensively, there are no estimates of the air-sea exchange of OC in the Southern Ocean. Here we examine the pools of EDOC and GOC and the associated air-water exchange of OC in the Antarctic Peninsula region, and compare this exchange to the flux of CO_2. We do so on the basis of three different cruises conducted along the Antarctic Peninsula in the austral summers of 2005, 2008 and 2009, onboard the R/V Hespérides.

Materials and methods

Three cruises were conducted onboard the spanish R/V Hespérides along the Antarctic Peninsula: ICEPOS (2 to 22 February, 2005), ESASSI (5 to 16 January, 2008) and ATOS-Antarctica (28 January to 23 February, 2009; Fig. 1). The ICEPOS and ATOS-Antarctica cruises, in 2005 and 2009, respectively, showed very similar trajectories around the Antarctic Peninsula, covering the portion of the Weddell Sea close to the Antarctic sound, the Bransfield Strait and the Bellingshausen Sea, while the ESASSI cruise, in 2008, was mostly restricted to the northern edge of the Weddell Sea, between the South Shetland and Orkney Islands (Figure 1). During ICEPOS, coupled

measurements of EDOC in surface waters and GOC were taken in 61 points along the cruise track, while only 20 and 25 coupled measurements were taken in ESASSI and ATOS-Antarctica, respectively. In addition to the coupled air-water measurements, depth profiles of EDOC concentration in the water column were performed in ICEPOS and ATOS-Antarctica. Additionally, several diel cycles and sampling of EDOC at the surface microlayer (SML) were conducted during ICEPOS only.

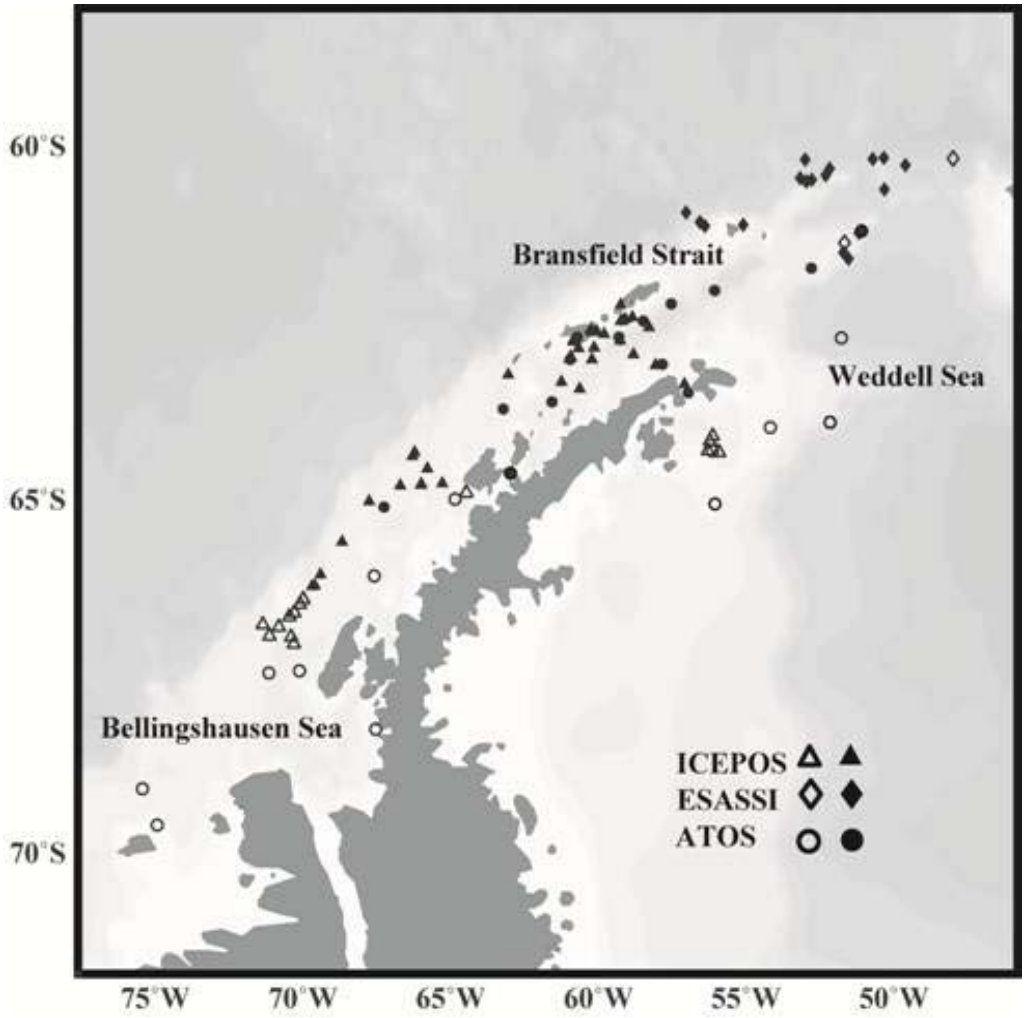

Figure 1. Map of the area of study and stations sampled during the three different cruises (triangles, ICEPOS (2 Fabruary to 22 febaruary 2005), diamonds, ESASSI (5 January to 16 January 2008 and circles, ATOS-Antarctica (28 January to 23 February 2009). open symbols correspond to stations undersaturated with regards to atmospheric CO_2, while closed symbols correspond to supersaturated stations.

CO_2 partial pressure was measured concurrently in air and water. XCO_2 in air was measured with a commercially available high precision (±1 ppm) non-dispersive infrared gas analyzer (EGM4, PP systems) passing clean air free of emissions from the vessel through an anhydrous Calcium sulfate (Drierite) column to remove water vapor and avoid interferences in the detector. XCO_2 in the sea was measured by circulating surface water, considering measurements taken at 5 m depth as surface measurements, the depth where the intake of the continuous flow-through system of the vessel is located. Water was pumped through a gas exchange column (1.25 x 9 membrane contactor, Celgard) and a closed-loop gas circuit fitted with an anhydrous calcium sulfate column was then circulated through the gas analyzer as above. The Continuous flow of water and the small volume of air circulating counter-current through the gas exchange column ensured full and rapid equilibration between water and air (Calleja et al. 2005). Partial pressure of CO_2 in water and air correspond to that in dry air (XCO_2), fugacity of CO_2 in water ($fCO_{2\text{-}w}$) and air ($fCO_{2\text{-}a}$) is then calculated by correcting for a 100% water vapor pressure at 1 atm pressure and applying the virial equation of state (Weiss 1974). The analyzer was calibrated using a commercial air mix of 541 ppm CO_2 and N_2 gas.

Samples for the analysis of EDOC and DOC were collected using Niskin bottles attached to a Rosette-CTD sampling system. Samples for dissolved organic carbon (DOC) analysis were collected in duplicate on 10 ml pre-combusted (4.5 h, 500 ºC) glass ampoules filled directly with sample water from the Niskin bottle, and acidified to a pH < 2 by adding 15 μL of concentrated (85%) H_3PO_4, sealed under flame and stored until analysis in the laboratory with a Shimadzu total organic carbon (TOC)-Vcsh, following standard non-purgeable organic carbon (NPOC) analysis (Spyres et al. 2000).

Standards provided by D. A. Hansell and W.Chen (University of Miami) of 2 μmol C L^{-1} and 44 μmol C L^{-1} were used to assess the accuracy of our estimates.

EDOC and GOC samples were collected following the procedure described in Dach's et al 2005 and Ruiz-Halpern et al. 2010. Briefly, for GOC samples, filtered air collected upstream from the boat to avoid contamination from in situ emissions, was bubbled for circa 30 min in 50 mL of high purity free of carbon miliQ water acidified to a pH<2 with concentrated (85%) H_3PO_4. EDOC samples were obtained by bubbling 1 L of sample water with High-grade (free of carbon) N_2 for 8 minutes, which we determined to suffice to reach equilibrium. The stream of gas with the evolved EDOC is redissolved in 50 mL of acidified, free of carbon Mili-Q water as for GOC H'^{-1}. Finally EDOC and GOC H'^{-} samples were stored in pre-combusted (4.5 h, 500ºC) glass ampoules sealed under flame until analysis in the laboratory as for DOC, but with the sparge gas procedure turned off in the Shimadzu total organic carbon (TOC)-Vcsh instrument (Dachs et al. 2005, Ruiz-Halpern et al. 2010). This effectively measured the EDOC trapped and the CO_2 contained in the water sample, which is subtracted following the analysis of blanks, obtained by directly bubbling the high purity acidified miliQ water with high grade N_2 without the sample water after collection of each set of EDOC and GOC at the stations.

Chlorophyll a (Chl a) concentration was determined spectrofluorimetrically (Parsons et al. 1984) for samples collected from Niskin bottles at various depths. The Chl a concentration was determined by filtering 50-mL samples onto 25-mm diameter Whatmann GF/F filters from each station. After filtration, filters were placed in tubes with a 90% acetone solution for 24 h to extract the pigment. Chl a fluorescence was measured in a Shimadzu RF-5301 PC spectrofluorimeter, calibrated with pure Chl a.

Krill abundance was estimated using a Simrad^{TM} EK60 multifrequency echosounder. Working frequency was 38 kHz with a 256 microsecond sampling interval, 1024 microsecond pulse duration and 2425 Hz bandwidth. The data obtained were processed using SonarData Echoview 4 software. A maximum depth of 100 m and 80 dB minimum target strength (TS), applying a time varied gain (TVG) function, was used to identify the krill targets. Finally the data were subjected to a 100 m depth cell and a 1 min duration analysis. The number of targets detected down to 100 m cells was counted at 1 min intervals, and the volume sampled by the beam calculated (Ruiz-Halpern et al. 2011).

Pressure, wind speed (U m s^{-1}), air temperature (Aanderaa meteorological station) fluorescence, sea-surface temperature (SST) and Salinity (PSU) (Seabird SBE 21 Thermo-salinographer) were measured continuously and averaged at 1 min intervals. Continuous fluorescence measurements were positively correlated with Chl a measurements taken at the stations ($r = 0.74$, $p < 0.05$), allowing the use of fluorescence as a proxy for phytoplankton abundance. Pitch, roll and heading of the research vessel were also recorded at 1 min intervals and used in a routine embedded in the software integrating navigation and meteorological data to correct wind speed for the ship movement and flow distortion. The corrected wind velocities were then converted to wind at 10 m (U10) using the logarithmic correction $U_{10}=U_z\ [0.097\ \ln(z/10) + 1]^{-1}$ where z is the height of the wind sensor position (Hartman and Hammond 1985).

Diffusive air-water exchange of CO_2 was estimated using the wind speed dependence of the mass transfer velocity (k600) from instantaneous wind speeds (U_{10}, m s^{-1}) following the equation $k600 = 0.222\ U_{10}^2 + 0.333\ U_{10}$ (Nightingale et al. 2000). The calculation of air-sea CO_2 flux (F_{CO2}) used the expression (eq 1):

(1) $F_{CO2} = k \times S \times \Delta fCO_2$

Where ΔfCO_2 is the difference between CO_2 fugacity in the surface ocean and that in the lower atmosphere ($\Delta fCO_2 = fCO_{2w} - fCO_{2a}$) and S is the CO_2 solubility term, calculated from water Temperature and Salinity (Weiss 1974). Likewise, organic carbon (OC) net diffusive fluxes (Faw) were estimated as the sum of gross volatilization (Fvol = k_0 x EDOC) and absorption (Fab = $-k_0$ x GOC H'^{-1}), where H´ is the dimensionless Henry's law constant and k_0 is the gas transfer velocity for exchangeable OC estimated from k600 values and Schmidt numbers assuming an average molecular weight (MW) of GOC of 120 g mol^{-1} and the same wind parameterization as for CO_2. Details for the associated uncertainties derived from the use of an average MW are given in Ruiz-Halpern et al. (2010), and details on the direct measurement of GOC H'^{-1} are given in Dachs et al. (2005). The ICEPOS cruise delivered 61coupled measurements of exchangeable organic carbon in water and air, while only 20 were obtained in ESASSI and 25 in ATOS-Antarctica (Figure 1). To characterize the stations sampled and compare CO_2 and exchangeable organic carbon fluxes hourly averages of SST, PSU, U, and $fCO_{2\text{-}w}$, $fCO_{2\text{-}a}$ and F_{CO2} were calculated during the ship work at station for each station where EDOC and GOC H'^{-1} estimates were collected.

Results

Wind conditions were variable, with values in excess of 20 m s^{-1}, but with low values close to 0 m s^{-1} detected in sheltered areas (Figure 2, Panel A). Seasurface temperatures were mostly around 0 ºC, although subzero temperatures were detected in the Weddell Sea close to the Antarctic Sound and the northeastern portion, and the southern Bellilngshausen Sea (Figure 2, Panel B). Salinity was less variable although values below 30 PSU were found in coastal areas receiving ice melt as well as the

southern Bellingshausen Sea and northeastern Weddell Sea (Figure 2, Panel C). Fluorescence showed maxima around the southern Weddell Sea, the Bellingsahausen Sea and the Bransfield Strait close to shore, indicative of the presence of blooms, and minima in the Weddell Sea and Bransfield Strait (Figure 2, Panel D). Mean seawater temperatures were close to 0ºC in all cruises, but always above (Table 1).

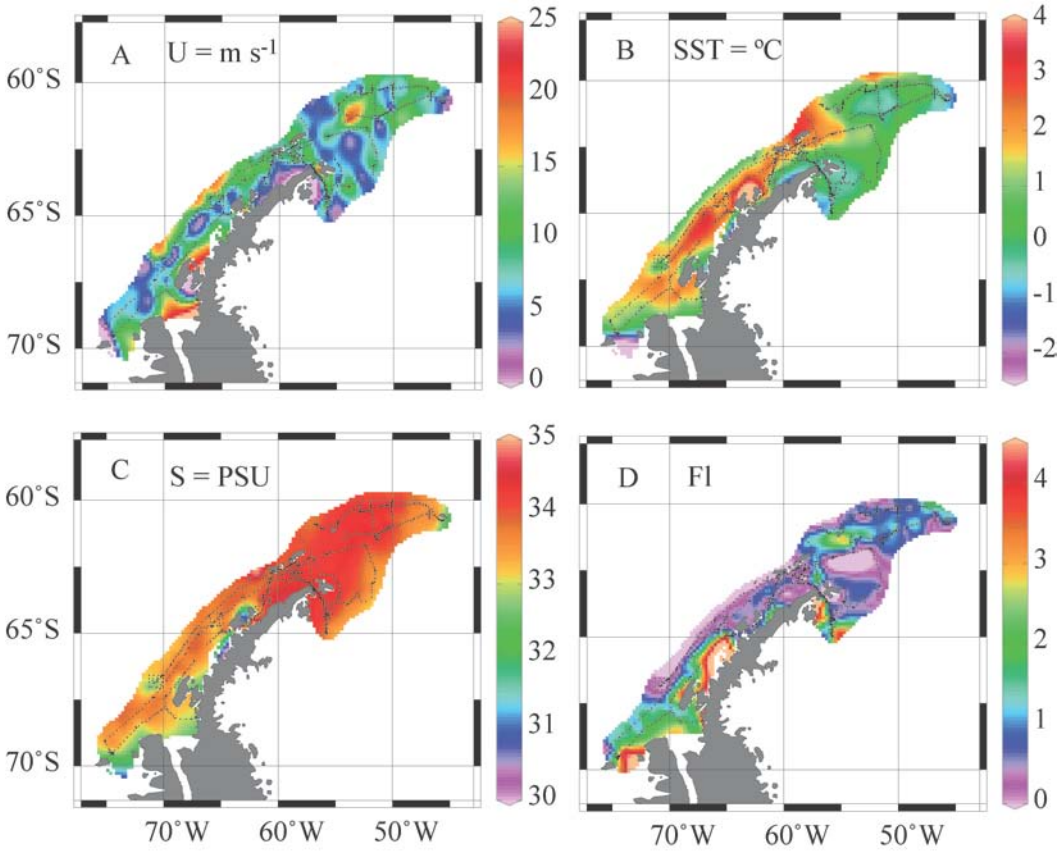

Figure 2. Gridded maps showing the combined area covered by all three cruise tracks (black dots) and shipboard continuous measurements of windspeed (m s^{-1}, A), seasurface temperature (ºC, B), salinity (PSU, C), Fluorescence (D).

However, temperature variation exceeded 4 ºC as a minimum of -1.1 ºC and a maximum temperature of 3.2 ºC were recorded both in the Bellingsahusen Sea during the ATOS-Antarctica cruise. Salinities were less variable, on average, the most saline water was found during the ESASSI cruise, mostly in the Weddell Sea (34.21), and the less saline water was located in the Bellingshausen Sea (33.35). However, a maximum salinity of 34.43 in ICEPOS at the Bransfield strait and a minimum of 32.53 PSU in the

Bellingshausen Sea during ATOS-Antarctica were recorded. Mean windspeeds were quite uniform across cruises and basins ranging between 6.6 m s^{-1} for ATOS and 8.18 m s^{-1} for ICEPOS.

Patterns in Chl a distribution were also highly variable with differences of up to 2 orders of magnitude in Chl a concentration among stations. The highest mean concentration of Chl a (3.91 mg Chl a m^{-3}) (Table 1) was found in the Weddell Sea during ATOS-Antarctica, while ESASSI had the mean lowest Chl a concentration (0.76 mg Chl a m^{-3}, Table 1). The lowest Chl a recorded was 0.12 mg Chl a m^{-3} in the Bellingshausen Sea during ATOS- Antarctica and an exceptionally high value of 31.66 mg Chl a m^{-3}, almost 300-fold the minimum value, was also measured during ATOS-Antarctica, but on the Weddell Sea (Table 1). Krill densities were generally low and the highest mean densities were located in the Bransfield Strait (Table 1). A maximum of 2.6 10^{-4} ind m^{-3} was found in the Bransfield strait during ICEPOS and a minimum of 1.9 10^{-5} ind m^{-3} in the Bellingshausen Sea during ATOS-Antarctica (Table 1).

The fugacity of CO_2 in surface seawater was also very variable with minima near shore, in the Bellingshausen Sea and southern Bransfield Strait, the southern Weddell Sea and northeastern tip (Figure 3, panel A), while the concurrent partial pressures in the atmosphere, $fCO_{2\text{-}a}$, were a lot less variable but displayed the opposite trend (Figure 3, panel B). ΔfCO_2 shows undersaturated areas along the coast and the Eastern and south Weddell Sea (Figure 1 and Figure 3, Panel D), while supersaturated areas were concentrated in the northern Weddell Sea and further from the coastline in the Bransfiedl Strait (Figure 1 and Figure 3, Panel D).

Table 1. Mean ± standard error (s.e), median and ranges, of hourly averages for the physical and biological parameters measured at the stations were coupled EDOC-GOC measurements were taken. Data fo rall three cruises; ICEPOS in 2005, ESASSI in 2008, and ATOS-Antarctica in 2009. Data has been divided into cruises and basins. Note that means for the different basins come from all three cruises and there was no acoustic data for the ESASSI cruise.

	SST	Sal	U	Chl a	krill density
cruise	ºC	PSU	m s^{-1}	mg m^{-3}	ind m^{-3}
ICEPOS	1.4±0.09	33.7±0.05	8.18±0.47	2.41±0.29	$8.5x10^{-5}$±$7.8x10^{-6}$
	1.7(-0.43-(+2.1))	33.8(32.74-34.43)	8.6(0.5-16.93)	2.2(0.55-4.58)	$7.2x10^{-5}$ ($2.7x10^{-5}$-$2.6x10^{-4}$)
ESASSI	0.32±0.13	34.2±0.04	7.4±0.69	0.76±0.1	n.d
	0.25(-0.47-(+1.46))	34.3(33.75-34.38)	7.4(1.7-11.9)	0.85(0.17-1.27)	
ATOS	1.31±0.24	33.8±0.09	6.6±0.5	3.91±1.37	$6.6x10^{-5}$±$6.3x10^{-6}$
	1.6(-1.1-(+3.2))	33.8(32.53-34.53)	6.1(2.73-11.58)	1.7(0.12-31.66)	$6.2x10^{-5}$ ($1.9x10^{-5}$-$1.6x10^{-4}$)

Basin					
Weddell	0.09±0.08	34.1±0.05	8.08±0.57	3.58±1.6	$5.9x10^{-5}$±$6.6x10^{-6}$
sea	0.02(-0.48-(+0.96))	34.2(33.36-34.39)	7.82(1.7-13.3)	0.86(0.17-31.66)	$6x10^{-5}$ ($2.7x10^{-5}$-$1.1x10^{-5}$)
Bransfield strait	1.61±0.09	33.9±0.03	8.07±0.58	2.59±0.41	$1.1x10^{-4}$±$1.1x10^{-5}$
	1.75(-0.17-(+2.76))	33.9(33.73-34.43)	8.19+(0.5-16.9)	2.4(0.33-7.7)	$8.1x10^{-5}$ ($2x10^{-5}$-$2.6x10^{-4}$)
Bellingshausen	1.54±0.81	33.3±0.32	6.64±2.6	1.74±0.71	$6.1x10^{-5}$±$5x10^{-5}$
sea	1.73(-1.1-(+3.2))	33.4(32.53-33.83)	6.34(2.4-12.7)	1.16(0.12-4.55)	$5.6x10^{-5}$ ($1.9x10^{-5}$-$1.6x10^{-4}$)
Total Mean ± s.e	1.17 ± 0.09	33.83 ± 0.04	7.6 ± 0.32	2.73 ± 0.64	$7.9x10^{-5}$±$5.6x10^{-6}$

The Flux of CO_2 (F_{CO2}) is consistent in pattern with the ΔfCO_2, although slightly modulated by the influence of windspeed in the flux calculations, displaying undersaturated areas with supporting oceanic CO_2 uptake mostly close to shore along the Bellingshausen and southern part of the Bransfield Strait and in the majority of the southern portion of the Weddell Sea and eastern tip of the northern Weddell Sea (Figure 3, panel C). On the contrary, the majority of the northern edge of the Weddell Sea as well as the areas furthest from shore of the Bransfield Strait and Bellingshausen Sea tended to be supersaturated, acting as a net source of CO_2 to the atmosphere (Figure 3, panel C).

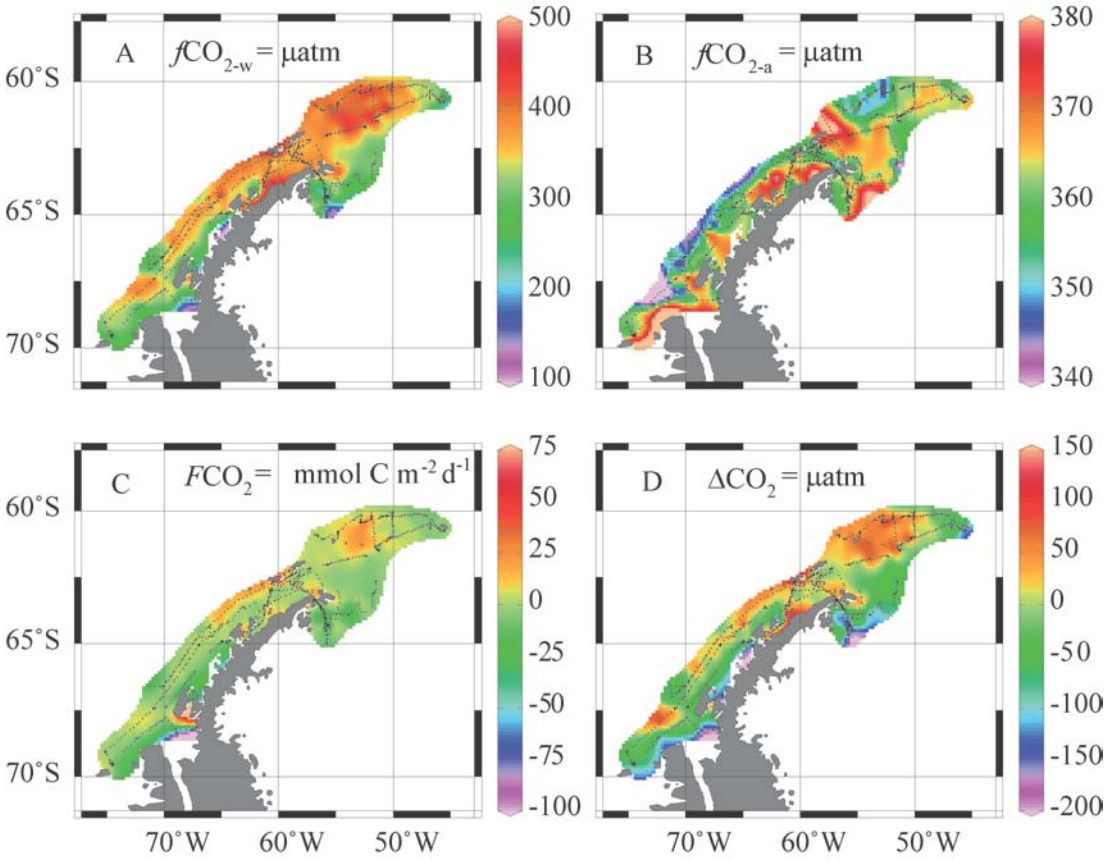

Figure 3. Gridded maps showing the combined area covered by all three cruise tracks (black dots) and shipboard continuous measurements of $fCO_{2\text{-}w}$ (µatm, A), $fCO_{2\text{-}a}$ (µatm, B), F_{CO2} (mmol C m^{-2} d^{-1},C), and ΔfCO_2 (µatm, D).

$fCO_{2\text{-}w}$ and $fCO_{2\text{-}a}$ were similar among cruises and regions, but $fCO_{2\text{-}w}$ was very variable (Table 2). With Surface water $fCO_{2\text{-}w}$ ranging from strong supersaturation in

the Bransfiel Strait during ICEPOS to a very strong undersaturation in the Weddell Sea during ATOS (Table 2). Supersaturation values above 400 µatm were found in all cruises and basins, while the Weddell Sea presented by far the most strongly undersaturated station (147.73 µatm), followed by the Bellingshausen Sea (281.9 µatm) and the Bransfield Strait, which was close to equilibrium (349.9 µatm, Table 2).

The maximum mean drawdown of carbon dioxide of 38.68 mmol C m^{-2} $d^{-1}$was found in the Weddell Sea during ICEPOS, while the maximum transfer of CO_2 to the atmosphere of 27.44 mmol C m^{-2} d^{-1} was calculated for the Bransfield Strait during the ICEPOS cruise (Table 3). There was a dominance of stations showing CO_2 uptake in the Bellingshausen Sea only, while CO_2 emissions prevailed in the Weddell and Bransfield strait and during all cruises. The fugacity of CO_2 in the water was positively correlated with Chl *a* (R^2=0.26, $p<0.05$) and negatively correlated with krill density (R^2=0.18, $p<0.05$), although these relationships were weak and driven by a few stations (Figure 6).

Surface water exchangeable organic carbon, the gaseous organic carbon fraction in the atmosphere and DOC, were, on average, similar among cruises and regions (Table 2). However, the variability of EDOC and GOC H'$^{-1}$ was high, ranging from virtually no EDOC present in surface waters at some stations to a maximum of 146.64 µmol C L^{-1} , with GOC H'$^{-1}$ ranging between 8.66 µmol C L^{-1} and 136.53 µmol C L^{-1} among stations (Table 2).Despite this variability, almost 60% of combined EDOC and GOC H'$^{-1}$ were comprised between 10 and 50 µmol C L^{-1} (figure 3). In contrast DOC values were less variable, except for two stations with values above 100 µmol C L^{-1}, mean ± SE DOC was 59 ± 3.8 µmol C L^{-1} (Table 2). Air water exchange of organic carbon exceeded the flux of CO_2 (Wilcoxon Sign-Rank test, $p<0.05$), by as much as a factor of 10 (Table 3), but this flux was not always in the same direction, and there was

a prevalence of ocean uptake of gaseous organic carbon among cruises and basins, except for the Weddell Sea where only 41% of stations had net fluxes towards the ocean, and ESASSI which was in close balance.

Table 2. Mean ± standard error (s.e) and ranges for partial pressure of CO_2 in water and air, EDOC, GOC H'$^{-1}$ and DOC throughout the three cruises ICEPOS in 2005, ESASSI in 2008, and ATOS-Antarctica in 2009. Data has been divided into cruises and basins. Note that means for the different basins come from all three cruises and there is no DOC data for the ESASSI cruise.

surface	fCO_2-w	fCO_2-a	EDOC	GOC H'$^{-1}$	DOC
cruise	µtam	µtam	µmol C L^{-1}	µmol C L^{-1}	µmol C L^{-1}
ICEPOS	368±10.03	356±0.71	36±3.66	35±2.84	54±1.40
	374(183-475)	357(345-374)	27(0-147)	29(11-134)	54(44.87-63.20)
ESASSI	396±8.48	357±1.44	40±7.71	43±9.11	n.d
	400(271-440)	358(345-366)	31(0.25-125)	34(9-136)	
ATOS	341±12.62	367±1.43	60±5.07	73±4.57	62±6.6
	364(148-416)	367(350-379)	57(0.85-102)	70(16-104)	54(45.1-181.37)
Basin					
Weddell sea	346±15.86	360±1.43	49±7.02	40±5.48	71±15.94
	387(148-440)	360(345-376)	42(0.25-147)	30(9-137)	56(48.15-1818.37)
Bransfield strait	401±6.21	360±1.03	39±5.05	50±3.53	54±1.37
	389(350-475)	359(350-379)	34(0.85-102)	44(20-134)	53(45.1-65.86)
Bellingshausen sea	344±7.15	356±1.38	41±5.79	47±7.80	58 ±5.14
	351(282-419)	353(346-372)	21(0-98)	21(11-100)	54(44.87-106.503)
Total Mean ± s.e	367 ± 6.66	359 ± 0.75	43 ± 2.96	46 ± 3.0	59 ± 3.81

In fact, the strongest sink for OC was found during the ESASSI cruise and reached 309 mmol C m^{-2} d^{-1}, and was mostly concentrated on the northern portion of the

Weddell Sea. The strongest source of OC from the ocean of 709 mmol C m^{-2} d^{-1} was found in the Weddell Sea during ICEPOS. These values are exceptionally high for net flux of OC and are related to high wind events at the time of sampling.

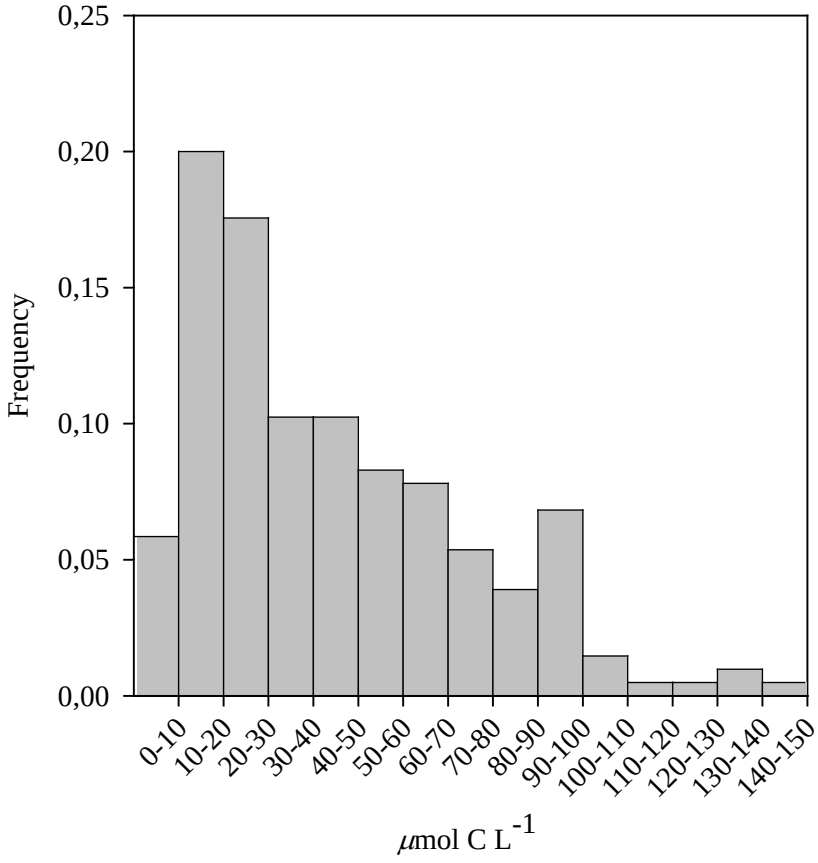

Figure 4. Frequency distribution of combined surface EDOC and GOC H'$^{-1}$ (μmol C L^{-1}) for all three cruises.

Moreover, gross diffusive fluxes between water and air of exchangeable organic carbon were much higher than the net flux (Table 3) but there were no significant differences between gross volatilization or gross absorption (Kolmogorov-Smirnov test, $p<0.05$). The flux of OC spanned a much broader range than that of CO_2 although two-thirds of the values were situated in a narrow band between -40 and 40 mmol C m^{-1} d^{-1} (Figure 4), while all fluxes of CO_2 from the stations were located in this band, with two thirds of the values representing net flux of CO_2 to the air (Figure 4), where the

Bransfiedl strait presented a 100% of supersaturated stations and only in the Bellingshausen Sea did undersaturated stations prevail over supersaturated ones (Table 3).

EDOC was independent ($p > 0.05$) of $fCO_{2\text{-}w}$, sea surface temperature or salinity, nor was there any relationship between EDOC or the Flux of OC with Chl *a* or krill density ($P > 0.05$). But there was a statistically significant positive correlation with GOC H'^{-1} ($p<0.05$), albeit with a low $R^2=0.1$ (Figure 7). During ICEPOS, the 11 stations, spanning most of the cruise track, sampled for surface microlayer-EDOC were significantly correlated with 5 m depth EDOC ($R^2=0.55$, $p<0.05$, figure 7) and the depth profiles of EDOC and Chl *a* in the water column performed during ICEPOS generally followed Chl a profiles (Figure 8, panel A). However, the 22 profiles performed during ATOS-Antarctica were more variable and not always in agreement with Chl a profiles. In fact, only 9 profiles followed Chl a concentrations (Figure 8, panel B), 10 showed no clear pattern with either a subsurface peak or a deficit in EDOC coincidental with subsurface Chl a max (Figure 8, panel C), and 3 were opposite to Chl a concentrations (Figure 8, panel D). The diel cycles performed during ICEPOS, from 3 February to 5 February, 13 February to 14 February and 16 February to 18 February 2005, showed, in general, two distinct peaks around midday and midnight for EDOC, while GOC H'^{-1} showed no evident diel pattern (Figure 9).

Table 3. Mean ± standard error (s.e), median and ranges for fluxes of organic carbon (Fvol, gross volatilization; Fab gross absorption; Faw, net air-sea exchange), and CO_2 (F_{CO2}) throughout the three cruises ICEPOS in 2005, ESASSI in 2008, and ATOS-Antarctica in 2009. Data has been divided into cruises and basins. Means from all basins come from all three cruises. And percent stations with undersaturated CO_2, and OC uptake

surface	Fvol	Fab	Faw	F_{CO2}	CO_2 uptake	OC uptake
cruise	mmol C m^{-2} d^{-1}	mmol C m^{-2} d^{-1}	mmol C m^{-2} d^{-1}	mmol C m^{-2} d^{-1}	% stations	% stations
ICEPOS	100±18.03 48(0-820)	-90.27±10.83 -70(-360-(0))	10±17.02 -6.6(-232-(+709))	1.37±2.01 2.3(-39-(+27))	20	56
ESASSI	95±33.6 35(0.6-556)	-115±37.87 -33(-606-(0))	-20±20.98 2.5(-310-(+101))	6.4±1.64 4.1(-5-(+21))	10	50
ATOS	106 ±19.51 72(1-385)	-130.84±22.97 -88(-457-(-24))	-27±11.43 -15(-161-(+63))	-1.99±1.35 0.05(-20-(+13))	46	71
Basin						
Weddell sea	135±35.18 72(0.6-820)	-115±26.78 -56(-606-(0))	20±30.72 6.3(-310-(+710))	-2.1±2.94 1.2(-39-(+21))	38	41
Bransfield strait	103±16.56 80(0-457)	-129±15.94 -109(-457-(0))	-27±12.22 -18(-232-(+224))	6.9±1.22 4.2.3(0-23)	0	72
Bellingshausen sea	66 ±12.67 41(0-293)	-64±13.46 -45(-386-(0))	2.24 ±12.16 -441(-161-(+150))	-1.5±0.78 -1.7(-9-(+6))	56	52
Total Mean ± s.e	101 ± 12.8	-105 ± 11.1	-4.9 ± 10.8	1.59 ± 1.17	28.3 ± 9.7	57 ± 5.5

Discussion

The physico-chemical and biological features of the Antarctic Peninsula reveal a highly dynamic area, where several water masses with distinct characteristics were encountered. Although in different years, the cruises took place in the same season. ICEPOS and ATOS were almost identical (dates and cruise tracks, Figure 1) and ESASSI took place a month earlier in the summer of 2008 and restricted to the Weddell Sea (Figure 1). Variability in the parameters measured is high, and particular stations may be considerably different from one another. However, this variation happens within cruises and basins, and total mean and median values for the cruises are remarkably similar, especially in regards to the physical characteristics, therefore it is reasonably safe to combine all the data.

The southern Bellingshausen Sea was, in general, colder and less saline. At the time of study this area had elevated Chl a values and bloom conditions in some stations were found. This feature was also present in some areas of the Weddell Sea close to the Antarctic Sound and on the northeastern tip close to the Orkney Islands. These colder, less saline waters are derived from the melting of the ice sheet during austral summer, as well as fresh water delivery form meltwater close to shore, and the accumulation of icebergs from the Weddell Sea in the Orkney Islands.

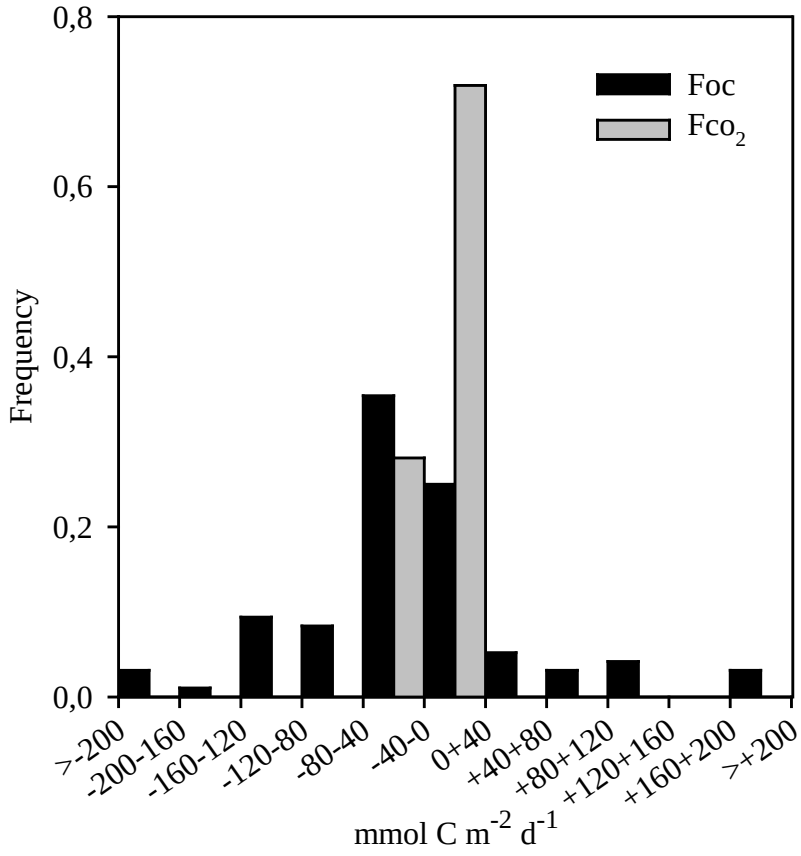

Figure 5. Frequency distribution of air-sea flux exchangeable organic carbon and F_{CO2} (mmol m^{-2} d^{-1})

Late spring and summer blooms are indeed controlled by abiotic factors as well as grazing pressure and are generally found in the marginal ice zone (Lancelot et al. 1993; Arrigo et al. 1998). The large variability in Chl a concentrations, spanning two orders of magnitude, corroborates the patchy nature in the distribution of phytoplankton found in Antarctic waters (El-Sayed and Klages 1994). Krill density also displayed a patchy distribution, in agreement with previous observations (Murray 1996), but the data presented here did not show any relationship to Chl a concentration, its main food source, as had been previously established in other studies (Atkinson et al. 2004).

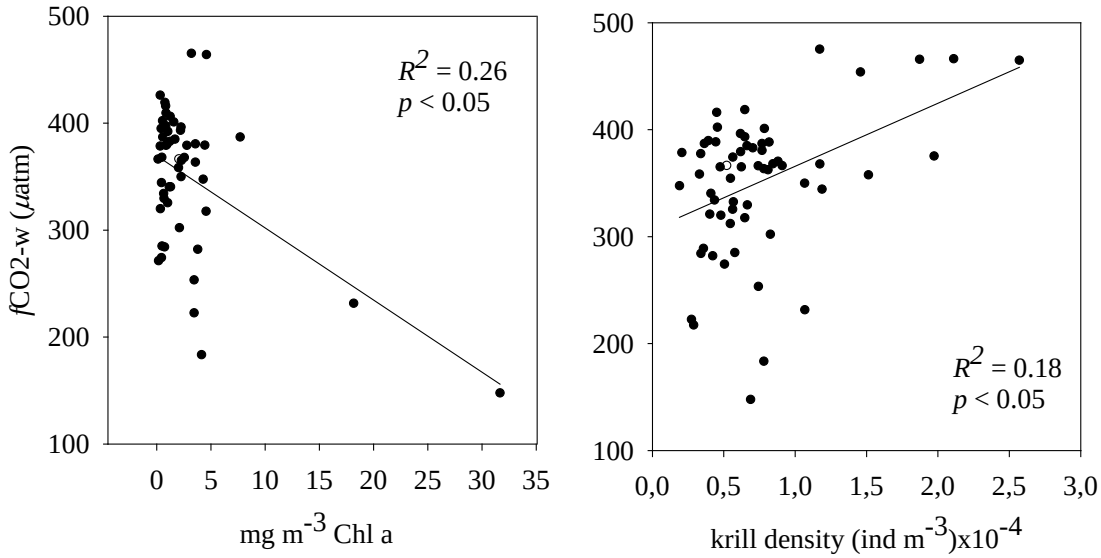

Figure 6. $fCO_{2\text{-}w}$ regressions vs Chl a (A) and Krill density (B). for both regressions $p<0.05$. Data combined for all cruises and stations

Mean EDOC and GOC H'^{-1} values were remarkably consistent with the values observed by Dachs et al. 2005 in the mid atlantic, (mean of 40 µmol C L^{-1}, and a range from 10 to 115 µmol C L^{-1}), highlighting the ubiqutous nature of this pool of carbon. The DOC values found in our study, overall mean surface DOC of 59±3.8 µmol C L^{-1} (Table 2) are in agreement with previous observations (Kähler et al. 1997) and makes the surface DOC pool in Antarctica rather small, smaller than values found in surface water of the Atlantic and coastal waters. Furthermore, in some cases DOC concentrations were lower than actual EDOC values. In fact, this low values of DOC render EDOC concentrations, similar to those found in other areas (Dachs et al. 2005, Ruiz Halpern et al. 2010) proportionately more important in Antarctic waters, since it amounts, on average, to 2/3 of the DOC present (from table 2), compared to 30-40% of DOC in the mid atlantic and subarctic regions (Dachs et al. 2005, Ruiz-Halpern et al 2010). This raises the possibility of an EDOC-dependent microbial community since these communities are often limited by carbon (Bird and Karl 1999) in these

environments. However, experiments on the utilization of VOC and SOC in oceanic waters are still lacking and this hypothesis remains speculative.

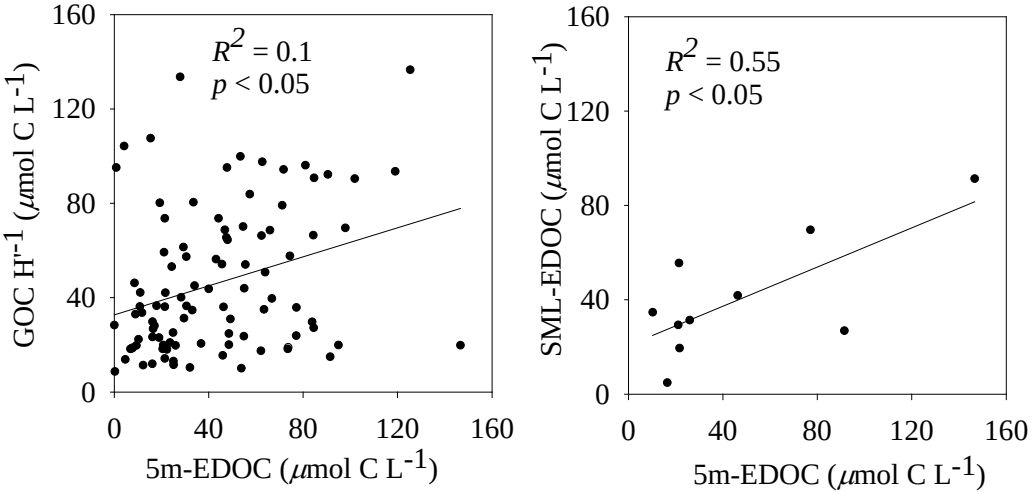

Figure 7. EDOC vs GOC H'$^{-1}$ regression ($p<0.05$) and, data combined for all cruises and basins. EDOC at 5m depth vs EDOC in the surface microlayer regression ($p<0.05$). Data for 11 stations during the ICEPOS cruise.

The linear relationship between atmospheric and dissolved exchangeable OC was not as consistent as previously demonstrated for the atlantic an subarctic regions (Dachs et al. 2005, Ruiz-Halpern et al 2010) indicating that other processes in the water column, or the atmosphere, control the relative partitioning of EDOC and GOC H'$^{-1}$. Reasons for this weak relationship may come from more intense UV radiation in the Antarctic peninsula (Madronich et al. 1998), affecting some of the volatile species present in the atmosphere, by photochemical degradation (Zepp et al. 1998) and reactions with OH radicals (Bunce et al. 1991), the degree of EDOC released by phytoplankton through UV induced cell lysis (Llabrés and Agustí 2010) and subsequent photochemical degradation in the water column, or rapid bacterial usage of EDOC (Villaverde and Fernández-Polanco 1999).

However, the positive relationship between 5 m depth EDOC and SML-EDOC, does provide indirect support of previous findings on the tight Air-Sea coupling of exchangeable organic carbon and its rapid diffusion across the water (Dachs et al. 2005; Ruiz-Halpern et al. 2010), where the fate of each gas will be determined by its intrinsic properties and concentration gradient. The variability of CO_2 concentration in the water was affected both by phytoplankton and krill. As a slow diffusing gas these relationships are not strong. However, they do allow the description of some of the mechanisms that influence CO_2 in the water. The stations where Chl a values were higher had the strongest undersaturation of CO_2, due to a strong photosynthetically mediated drawdown of carbon. Likewise, krill density influenced $fCO_{2\text{-}w}$ in the opposite direction as phytoplankton. Indeed, Krill not only consume phytoplankton, hence decreasing carbon fixation rates by algae, they also remineralize organic matter back to CO_2 through respiration (Mayzaud et al. 2005). As a result of the spatial heterogeneity in Chl a and Krill, CO_2 in the water was also very variable. Interestingly, $fCO_{2\text{-}a}$, although less variable (less than 30 µatm range), had the opposite distribution as $fCO_{2\text{-}w}$ (Figure 2, panels A & B), indicating that the ocean may exert small spatial scale influence in the concentration of CO_2 in the atmospehere, a possibility that has yet to be explored, and warrants further investigation, since most global estimates of air-sea flux of CO_2 are based on regional mean atmospheric values over large areas (Takahashi et al. 1997; Takahashi et al. 2009).

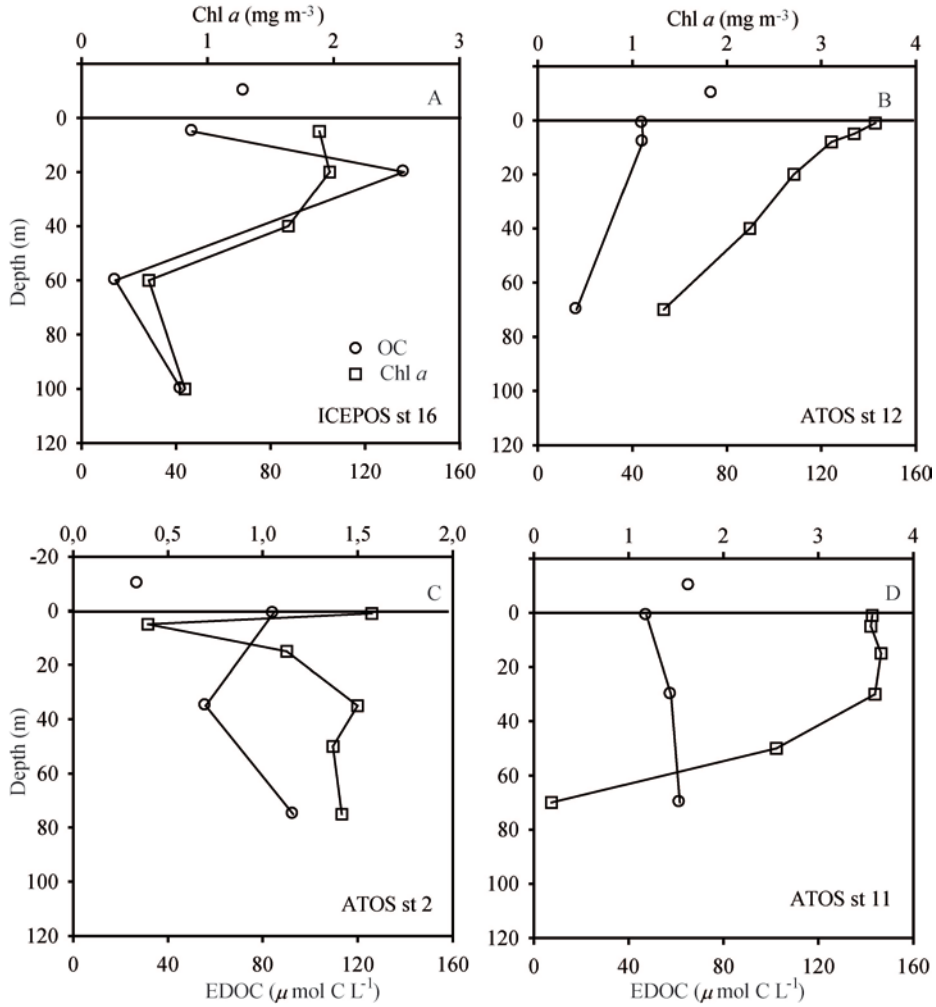

Figure 8. Depth profiles of EDOC and Chl a. Only 3 representative types of profiles are shown. The 5 profiles of performed during ICEPOS were all similar (A). During ATOS-Antarctica, 9 followed Chl a (B), 10 showed no clear pattern (C), and 3 were opposite to Chl a (D). Open circles (EDOC), single open circle (GOC H'$^{-1}$), open squares (Chl a).

The direction and potential intensity of carbon dioxide flux is determined by the gradient difference between air and water (ΔfCO_2). However, wind speed modulates this intensity with higher wind speeds producing larger fluxes. This spatial heterogeneity, together with the high variability of the wind fields in Antarctica obscures the characterization of the Antarctic Peninsula as a source or sink for CO_2. In fact, F_{CO2} data from the hourly averages at the stations (Table 3, Figure 5), point to a prevalence of net efflux of CO_2 to the atmosphere, somewhat in disagreement with the

overall continuous mapping provided in figure 3, since the overall mean suggests the Antarctic Peninsula as a small source of CO_2, with most stations being supersaturated with regards to the atmosphere (Table 3, figure 5), while figure 3 (panel C) shows the Antarctic Peninsula to be a moderate sink for CO2, in better agreement with Gruber et al. (2009) who pointed to the Antarctic Peninsula as a weak global sink for CO_2. This discrepancy between discrete and continuous measurements in highly variable and biologically active areas, highlights the importance of the acquisition of high resolution (both spatially and temporally) data on CO_2. On the other hand, even if only data for the stations is currently available since sampling for EDOC and GOC is, as of yet, not possible to be undertake in a continuous or semi-continuous way, the flux of OC was predominantly towards the ocean (Table 3, Figure 5), which corroborates the ocean as a global sink for VOC and SOC (Dachs et al. 2005, Ruiz-Halpern et al. 2010). However, a large portion of the stations had a net export of OC to the atmosphere. This dual source sink nature for VOC and SOC would support the redistribution of
Organic carbon within the ocean supplementing that mediated by water mass transport, as pointed out by Dachs et al. 2005.

The depth profiles of EDOC revealed the active nature and participation of phytoplankton in the production of exchangeable organic carbon, which is consistent with the size dependent release of Volatile Organic carbon demonstrated by Ruiz-Halpern et al (submitted) and as a source of DOC to the environment (Ruiz-Halpern et al 2011). The profiles also suggest active consumption in the watercolumn and other players, such as bacteria, may consume EDOC, since bacteria are often limited by carbon supply, as the DOC pool in Antarctic waters is particularly small (Kähler et al. 1997). Moreover, Krill has been recently demonstrated to release large amounts of DOC and EDOC available to the microbial community (Ruiz-Halpern et al. 2011, this thesis),

and it is not unlikely that it may also contribute to the EDOC pool, either mechanically, by ruputure of phytoplanktonic cells during sloppy feeding (Moller 2007) or via direct excretion (Ruiz-Halpern et al. 2011).

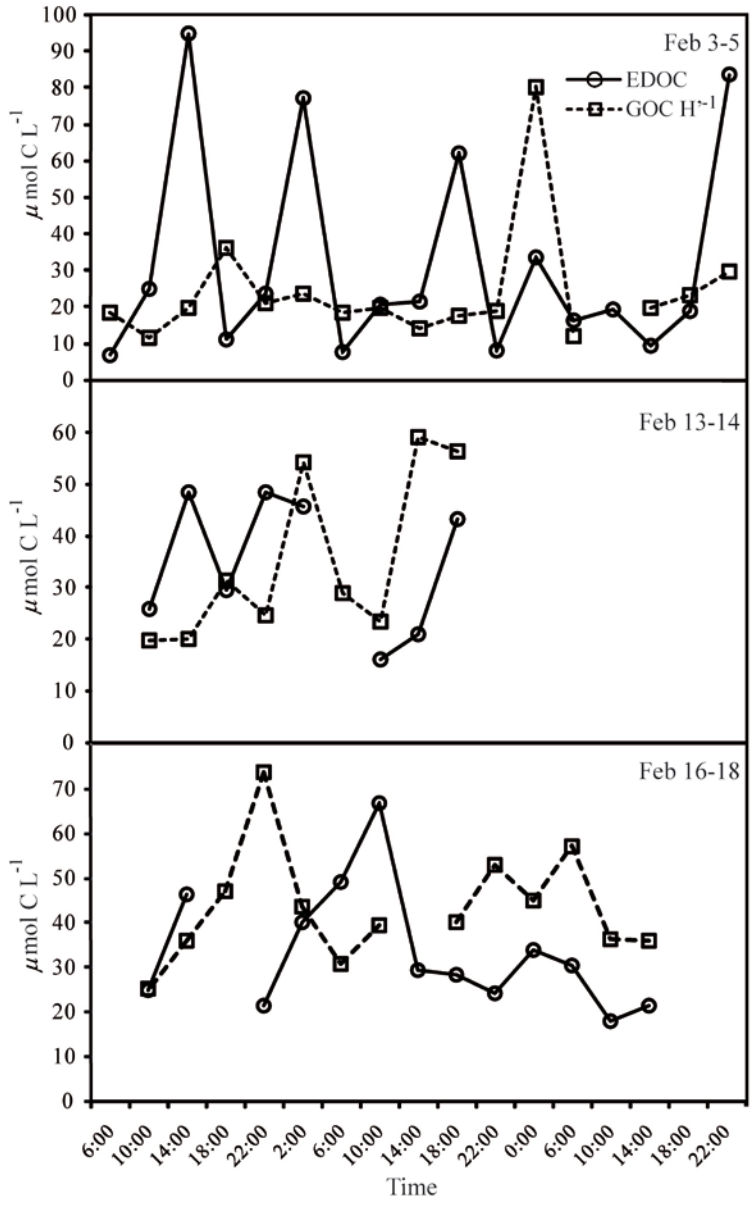

Figure 9. Diel cycles of EDOC (solid line, open circles) and GOC H'$^{-1}$ (dashed line, open squares).

The diel cycles performed, provide further evidence of the dynamic nature of EDOC in the water column, since peak EDOC concentrations were detected both at midday and midnight, while GOC H'^{-1} was less variable. These cycles point to a progressive build up of EDOC in the water column as the day progresses, phytoplankton receive more light (both PAR and UV) photosynthesis proceeds, and UV damage becomes larger, and another peak in night time that could be related to Krill's vertical migration patterns with an initiation of an upward motion at dusk (Zhou and Dorland 2004), and subsequent release of EDOC by sloppy feeding or excretion (Moller 2005), a hypothesis that awaits further experimental tests.

The data gathered during the three cruises in the Antarctic region, provide compelling evidence, and gives further support from previous findings (Dachs et al. 2005; Jurado et al. 2008; Ruiz-Halpern et al. 2010) that carbon biogeochemistry research in the oceans may be missing an important pool of carbon, ubiquitous and highly reactive in nature, with unforeseen important consequences for the global carbon budget and biogeochemical interactions in the ocean and the atmosphere. Additionally, evaluation of the EDOC pool in antarctic waters reveals this pool to be comparatively more important than in other regions since it accounts for a larger proportion of total OC, with values higher than DOC in some instances. Furthermore, this exchange of carbon, comparable in magnitude to the flux of CO_2, is made up of a mixture of gases, most of them unknown, and may present a suite of effects in the chemistry of the atmosphere, affecting the radiative balanace, green house effects, oxidative properties of the atmosphere, hydroxyl radical formation, ozone depleting phenomena in the stratosphere and ozone formation in the lower atmosphere, or cloud and secondary aerosol formation. Likewise, little is known on the properties and possible effects of EDOC in the water column, and other possible producers other than micro and

macroalgae. In fact, bacteria also produce (Kuzma et al. 1995) and consume (Cleveland and Yavitt 1998) VOC as has been demonstrated for the production and consumption of isoprene, and could contribute, not only to the EDOC pool in the water column, but also to its turnover. Indeed, the molecular weight of volatile and semivolatile species is low compared to DOC, and it is likely that this molecules are eagerly demanded by the microbial community as a fast an easy source of carbon, greatly contributing to respiratory processes in the ocean.

In summary, the data presented here, only fills a minor gap in the huge paucity of data and lack of knowledge regarding the biogeochemical cycling of volatile and semivolatile species of carbon in the Antarctic, and other oceans and coastal areas alike, and calls for the need of a global concerted effort to expand the number of observations in a wide variety of environments, and perform experiments where biogeochemical interactions involved in the cycling of this carbon can be identified and key processes explained. This article highlights the important role, to date mostly overlooked, of this pool of carbon in the ocean.

Acknowldegments

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Es característico de la vida,

la falta de permanencia de las estructuras materiales.

Aunque la cantidad total permanezca semejante a sí misma,

algo entra y algo sale.

Ramón Margalef

(1919-2004)

Chapter 3

Antarctic krill as a source of dissolved organic carbon to the Antarctic ecosystem.

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Size-dependence of volatile and semi-volatile organic carbon content in phytoplankton cells

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Antarctic krill as a source of dissolved organic carbon to the Antarctic ecosystem

Abstract

The role of krill as a source of dissolved organic matter in the Southern Ocean was tested through a series of experiments performed around the Antarctic Peninsula. These experiments revealed high, but variable release rates of dissolved material (carbon and nutrients), supplying, on average 150 mmol dissolved organic carbon (DOC) m^{-2} d^{-1}, which is comparable to that supported by phytoplankton. Krill support, on average, 73% of the combined krill + phytoplankton production of dissolved organic carbon in the ecosystem, implying the importance of krill in conditioning the productivity of the Southern Ocean. However, the contribution of krill as a source of DOC varied greatly, due to the patchy distribution of both krill and primary producers in the region, ranging from 98 % to 10 % of the combined (krill + phytoplankton) DOC release rates. These results suggest that rapid decline in krill standing stocks associated with reduced ice cover may have major consequences for microbial communities in the ecosystem, since bacterial carbon demand (BCD) often exceeds the dissolved organic carbon supplied by phytoplankton in coastal areas of the Southern Ocean, with potential unforeseen consequences in the carbon balance of the Southern Ocean.

Introduction

Antarctic krill, *Euphasia superba* - with an estimated abundance of ~170 x 10^9 kg (Atkinson et al. 2006) is arguably one of the most abundant animals on Earth (Nicol

and Endo 1997; Siegel 2005), acting as a major node in the Antarctic food web, where it is consumed by a broad range of Antarctic megafauna (Murphy 1995). Provided its high biomass and production, krill must also play a key role in the recycling of materials, thereby affecting the Antarctic microbial food web both as a consumer (Atkinson et al. 2001) and as a source of necessary recycled materials (Tovar-Sanchez et al. 2007). Indeed, available evidence shows that krill plays a crucial role in recycling nitrogen, phosphorus, iron and other metals in the Southern Ocean, contributing to the high primary productivity of these waters by supplying readily utilizable iron, phosphate and ammonia (Ikeda and Mitchell 1982; Tovar-Sanchez et al. 2007; Tovar-Sanchez et al. 2009). Krill has been recently shown to play a key role in producing chromophoric dissolved organic matter (Ortega-Retuerta et al. 2009), providing indications that krill can play a significant role as a source of dissolved organic carbon (DOC) to the environment. Previous attempts to use Chromophoric dissolved organic carbon (CDOM) measurements to infer DOC concentrations yielded adequate correlations, only when DOC dynamics are controlled by terrestrial or riverine inputs. Moreover, because the mechanisms that drive CDOM and DOC distribution are uncoupled in oceanic areas, no conversion factors found in the literature can be used to estimate DOC from CDOM in the Southern Ocean (Hanselll and Carlson 2002; Coble 2007). Hence, quantitatively assessing the role of Antarctic krill as a source of DOC to the microbial food web is particularly important, because bacterial communities in Antarctica are quite sparse relative to the biomass of autotrophs compared to other ocean areas (Duarte et al. 2005). Possible explanations for the low abundance, and activity, of bacteria in the Southern Ocean include substrate limitation (Church et al. 2000; Pomeroy and Wiebe 2001) resulting in low growth rates that render bacterial communities prone to grazer control (Duarte et al. 2005). Evaluations of DOC release by Antarctic phytoplankton

communities showed that release rates vary between 3% to 47% of primary production (Morán and Estrada 2002). These values fall within the range of those reported for other oceans, although it is not uncommon to find higher values (Karl et al. 1998; Myklestad 2000; Teira et al. 2003). This release of DOC by phytoplankton is generally found to be coupled to bacterial production in the southern ocean (Morán et al. 2001). However there is also evidence of decoupling between bacterial and phytoplankton production in coastal areas (Morán et al. 2002), such as the Gerlache strait (Bird and Karl 1999).

It is well established that there are a suite of mechanisms that can lead to DOC release into the water column, providing the necessary DOC to sustain carbon consumption rates, when these exceed primary production (Nagata 2000). Grazers (protozoa and zooplankton) contribute to the DOC pool by sloppy feeding, excretion, egestion and dissolution of fecal pellet material (Strom 1997). Viral infections of bacteria and phytoplankton can also compose an important fraction of the DOC released to the environment (Nagata 2000). Protozoans account for as much as 50% of total DOC, while zooplankton produce as much as 12% (Nagata, 2000). Several experiments demonstrate the importance of copepods in the production of DOC (Strom et al 1997; Urban-Rich 1999; Moller 2007). However, to the best of the author´s knowledge, there are no quantitative estimates of DOC contributed by zooplankton in the Antarctic peninsula. As one of the most abundant species in this environment, Krill must then play a fundamental role in supplementing BCD above the limits imposed by DOC directly deliverd by autotrophs. Therefore, its role as a source of DOC in the Southern Ocean ecosystem has not yet been elucidated.

Here we evaluate experimentally the rates and dynamics of DOC and nutrient release by Antarctic krill, as the major zooplanktonic species in this environment (Ross et al. 1996), and their stoichiometry with nitrogen and phosphorus, and evaluate the

importance of krill-derived DOC relative to the DOC produced by phytoplankton. We do so on the basis of a cruise, Aportes Atmosféricos de Carbono Orgánico y Contaminantes al Océano Polar (ATOS)-Antarctic, conducted on board the R/V *Hespérides* around the Antarctic Peninsula.

Methods

The release of DOC, NH_4^+, total nitrogen (TN), and total phosphorous (TP) by Antarctic krill (*Euphausia superba*) was examined between January and February 2009, time of peak krill abundance (Siegel 2000) during the ATOS-Antarctic cruise on board the R/V *Hespérides* along the Antarctic Peninsula sector of the Southern Ocean. All experiments were done along the Antarctic Peninsula, including the Weddell Sea, the Antarctic Sound, the Bransfield Strait, down to the Bellingshausen Sea, south of the Polar Circle (Fig. 1). Krill individuals to perform excretion experiments were collected within the upper 50 m at 8 stations with contrasting oceanographic characteristics (Fig. 1). Krill Swarms were located using a Simrad^{TM} EK60 multifrequency echosounder. The specimens to perform the experiments were caught using an Isaacs-Kidd Midwater Trawl (IKMT) net (1cm mesh size) trawled for 20 min at the depth where krill swarms were located (typically about 20-30 m depth). Krill specimens were caught whenever a large enough swarm was identified, and as such, the time of day for the experiments varied among the different dates (Table 1). Time of day of experiments was not expected to affect krill feeding rates, since they feed continuously in the field (Antezena et al. 1982; Morris et al. 1983).

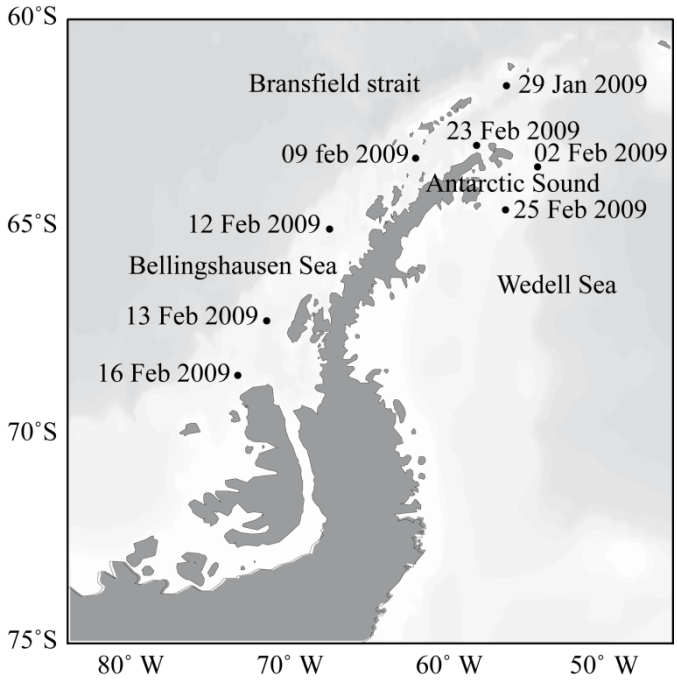

Figure 1. Location and date of sampling of the 8 experimental stations spaced along the Antarctic peninsula.

After retrieval, live krill were placed in a 50-L plastic container filled with seawater and held for a short time (typically less than 5 minutes) before the experiments were performed. The release experiments followed the procedure described by Tovar-Sanchez et al. (2007). Before the krill was captured, surface seawater (1 m depth) was pumped from a small boat through a Cole Parker peristaltic pump using acid-cleaned teflon tubing coupled to C-flex tubing, filtered through an acid cleaned polypropylene cartridge filter (0.22 mm, MSI-Calyx®), and collected in 2 L bottles for the release experiments. Four randomly selected krill individuals were transferred into each of 3-5 acid-washed 2 L polycarbonate bottles, so that 12 to 20 individuals were used per station, with a total of 144 individuals tested along the cruise. Experimental (containing krill) and control bottles (without krill) were incubated in the dark, at in situ temperature, for 30, 60, 90, 120, and 240 minutes. At each time step water samples were

collected from a different bottle and analyzed for DOC, NH_4^+, TN, and TP. Krill were then retrieved and kept frozen until analyzed in the laboratory, where specimens were thawed, dried to constant weight at 60ºC in a drying oven and weighted to the nearest mg. For DOC, water was filtered through GF/F filter and 10 mL aliquots transferred to duplicate glass ampoules, precombusted at 450ºC for 5 hours, sealed under flame and stored until analysis in the laboratory. DOC analyses were performed on a Shimadzu Total Organic Carbon (TOC)-5000 or TOC-Vcsh following high temperature catalytic oxidation techniques (Spyres et al. 2000). Standards provided by D. A. Hansell and W. Chen (University of Miami) of 2 μmol L^{-1} and 44 μmol L^{-1} TOC were used to assess the accuracy of the estimates.

Water for TN and TP analysis was collected in duplicate in high density polyethylene (HDPE) tubes and kept frozen until analyzed in a Bran-Luebe AA3 autoanalyzer using standard methods (Hansen and Koroleff 1999) with ultra violet (UV)-mineralization (Oms et al. 2003). NH_4^+ concentrations were determined spectrofluorometrically onboard using a Shimadzu spectrofluorimeter (Kérouel and Aminot 1997). Krill abundance was estimated using a Simrad^{TM} EK60 multifrequency echosounder. Working frequency was 38 kHz with a 256 microsecond sampling interval, 1024 microsecond pulse duration and 2425 Hz bandwidth. The data obtained were processed using SonarData Echoview 4 software. A maximum depth of 100 m and 80 dB minimum target strength (TS), applying a time varied gain (TVG) function, was used to identify the krill targets. Finally the data were subjected to a 100 m depth cell and a 1 min duration analysis. The number of targets detected down to 100 m cells was counted at 1 min intervals, and the volume sampled by the beam calculated. Krill biomass was estimated as the product of the individual weight (wt) of krill in each station and the density (individuals m^{-3}) of krill within 10 m depth bins, and vertically

integrated within the top 50 m of the water column, the layer containing most of the specimens.

In situ primary production, total and particulate, was measured by the ^{14}C technique (Steeman-Nielsen 1952) as described in Lasternas and Agustí (in press). Water was sampled at least from 3 depths including the surface (1 m), the subsurface (5 m) and the deep chlorophyll maximum (DCM), in all cases shallower than 50 m. Water collected at these depths was transferred into transparent (light) and dark 150 mL polycarbonate bottles, and inoculated with 100 μCi activity of a ^{14}C working solution. Inoculated bottles were suspended at the corresponding depths from a drifting buoy and incubated in situ for 4 hours. At the end of the incubation period duplicate 5 mL aliquots were transferred into 20 mL scintillation vials for the determination of total primary production production (TPP). The remaining volume was filtered through 0.22 μm mesh membrane filters (cellulose membrane filters) of 25 mm diameter to determine particulate primary production (PPP > 0.22 μm). Samples were acidified with 100 μL of 10% HCl and shaken for 12 h to remove inorganic ^{14}C. Then, 10 mL of scintillation cocktail (Packard Ultima Gold XR) were added to TPP vials and the disintegrations per minute were counted after 24 h with a scintillation counter (LS 5801, Beckman). DOC production by phytoplankton was calculated as the difference between total and particulate primary production (Morán et al. 2001). The incubations contain the full microplankton community and, hence, the DOC release measurements include contributions from protist grazing and viral lysis as well (Nagata 2000). However, for simplicity we refer to these estimates as phytoplankton DOC release.

Chlorophyll *a* concentration was determined by filtrating 50 mL samples onto 25 mm diameter Whatmann GF/F filters from each depth at each station. Following filtration, filters were placed in tubes with a 90% acetone solution for 24 hours to

extract the pigment. Chlorophyll *a* fluorescence was measured in a Shimadzu RF-5301 PC spectrofluorimeter, calibrated with pure Chlorophyll *a* (Chl *a*) as described in Parsons et al. (1984).

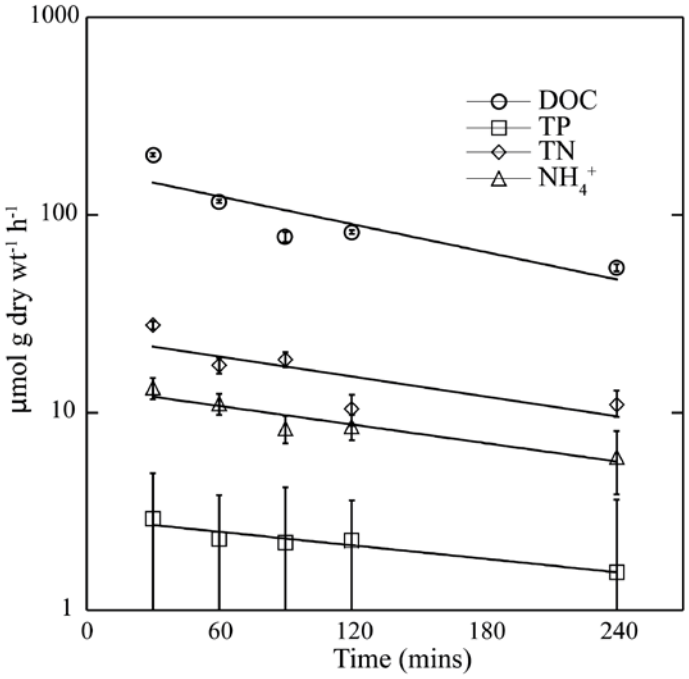

Figure 2. Fit of the geometric mean±SD release rates for the 8 experiments. DOC=170 $e^{-0.0054time}$, R^2=0.75. TN=24 $e^{-0.0039time}$, R^2=0.68. TP=2.9 $e^{-0.0026time}$. R^2=0.88. NH_4^+=13 $e^{-0.0036time}$. R^2=0.89. All p<0.05 except TN p>0.05

Table 1. Integrated DOC pool in the water column (0 to 50 meters), phytoplankton production (POC=particulate, DOC=dissolved), krill biomass and DOC release by krill for each of the 8 stations where release experiments were conducted. Daily production was calculated at 14 h for phytoplankton and 24 h for krill, using the release rate calculated from the first 30 minutes of incubation from each experiment.(SE = standard error, nd: no data)

	Water Column	Phytoplankton				Krill	
experiment	DOC (mol m^{-2})	POC (mmol C m^{-2} h^{-1})	DOC (mmol C m^{-2} h^{-1})	DOC (%TPP)	Chl *a* (mg m^{-2})	g dry wt m^{-2}	DOC (mmol C m^{-2} d^{-1})
29 Jan 09	2.91	148.11	126.22	46.01	69.91	1.98	0.59
02 Feb 09	2.91	18.23	34.84	65.65	28.40	13.77	1.58
09 Feb 09	2.62	57.84	89.44	60.73	121.64	34.50	6.59
12 Feb 09	2.77	10.95	7.43	40.43	12.07	59.38	15.56
13 Feb 09	2.92	53.07	52.56	49.76	46.63	13.25	nd
16 Feb 09	2.31	nd	nd	nd	40.81	27.70	6.93
23 Feb 09	2.43	32.87	3.15	57.33	32.83	nd	nd
25 Feb 09	3.05	12.58	2.39	72.65	41.86	nd	nd
mean	2.74	47.66	45.15	56.08	49.27	25.10	6.25
SE	0.10	19.64	19.52	4.64	11.87	9.11	2.97

Results

The experiments conducted at 8 different stations along the Antarctic Peninsula (Fig. 1) showed that krill release large amounts of DOC and nutrients, although the release rates decline rapidly with time (Fig. 2). Krill released two-thirds of the total DOC released along the full length of the incubations within one 1.5 h after the onset of the experiment (Fig. 3). The geometric mean initial rate of DOC release was 202 μmol C g dry weight (dry wt)$^{-1}$ h^{-1} (table 2), although this rate was highly variable among experiments (Table 1). Nutrient release rates followed the same trend as those for DOC release (Fig. 2, Table 2). The initial excretion products released by krill were relatively nutrient-depleted, as evidenced by high C:N and C:P ratios (median 73.93 and 140.36, respectively), with the C:N ratio declining along the course of the incubations (Table 3).

Phytoplankton primary production integrated down to 50 meter was highly variable across the stations sampled, ranging from 18.4 to 274.3 mmol C m^{-2} d^{-1}, with a mean (± SE) DOC percent extracellular release (PER) of 56.08 ± 4.64 % (Table 3) of total primary production (TPP). Particulate organic carbon (POC) and DOC production profiles showed variable rates, spanning two orders of magnitude with a peak at the DCM except for 12 February 2009 (Fig. 4), where a large bloom occurred (Table 1). Krill standing stock averaged 25.1 ± 9.1 g dry wt m^{-2} and was calculated to release on average 150 ± 71.3 mmol C m^{-2} d^{-1} as DOC. Chl *a* standing stock averaged 49.27 ± 11.87 mg C m^{-2}, with an average release of DOC of 55.44± 16.26 mmol C m^{-2} d^{-1} (Table 3). However, only two of the four stations were both rates could be calculated did Krill DOC release exceed total primary production (Table 1). The combined flow of DOC from phytoplankton and krill averaged 205.4 ± 77.7 mmol C m^{-2} d^{-1} compared

to an average DOC standing stock of 2.7 ± 0.1 mol C m^{-2} (Table 1).

Table 2. Geometric mean and geometric standard deviation (SD) of DOC and nutrient release rates by Antarctic krill in μmol g dry wt^{-1} h^{-1} (n = 8 experiments). The rates reported are those calculated after 30 minutes of incubation.

	mean	SD
DOC	202.00	4.15
TN	27.72	1.35
TP	2.92	2.05
NH_4^+	13.35	1.66

Discussion

The data presented here highlights the important role that krill plays in the Southern Ocean. Indeed, krill is recognized as the central node in the Antarctic ecosystem, providing a food source for higher order consumers (whales, penguins, seals) (Murphy 1995), but also remineralizing nutrients and metals essential for phytoplankton growth (Ikeda and Mitchell 1982; Tovar-Sanchez et al. 2007). The results demonstrate that krill also play a key role as a source of DOC, thereby increasing the flow of carbon potentially available to support heterotrophic bacteria. Recent evidence that krill release substantial amounts of colored dissolved organic matter, with doubling times of less than a day at high krill densities (Ortega-Retuerta et al. 2009) provided indications for potentially high DOC release.

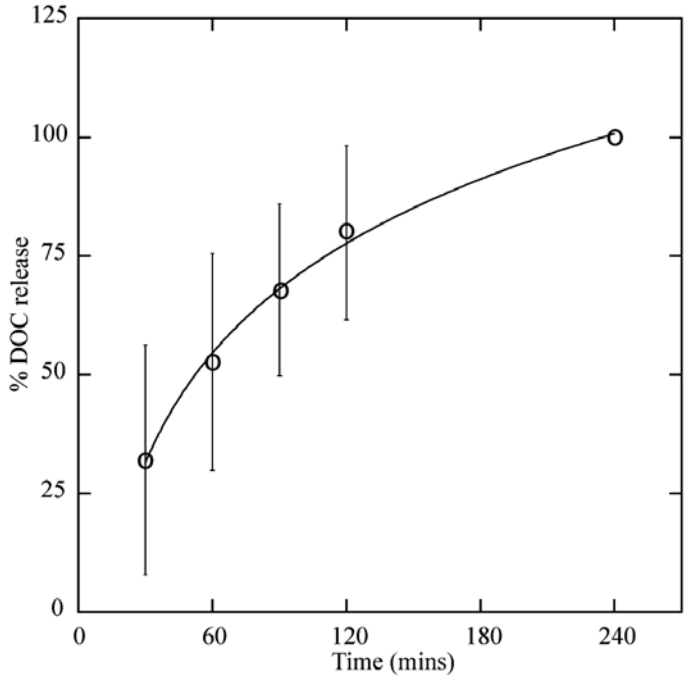

Figure 3. Mean ± SD percent of total DOC released at each time step, calculated from the release rate of each experiment at each time step.

Yet, the use CDOM as a proxy for DOC is only supported when riverine discharge controls the distribution of both DOC and CDOM. Attempts to calculate DOC from CDOM in other environments are flawed by large uncertainties, largely due to decoupled dynamics of both fractions, and a variable background of non-coloured DOC in the water (Coble 2007). The results presented here, quantitatively demonstrate that krill plays an important role in the flux of dissolved organic matter.

As demonstrated for nutrient and metal release (Tovar-Sanchez et al. 2007, 2009), the release of DOC by krill decreased rapidly over time when the animals are removed from prey, as was the case for the experiments conducted here. This is in agreement with the reported krill gut clearance rates of < 1 h (Morris et al. 1983; Clarke et al. 1988). As krill are constantly feeding (Morris et al. 1983), they are able to

maintain high DOC release rates throughout the day, releasing, on average, 16% of their carbon biomass as DOC per day (calculated from the data in Table 1). The depletion in N and, to a lesser extent, P relative to DOC of krill release products (Table 3) could limit bacterial use in the absence of inorganic nutrients. However, inorganic nutrient concentrations are characteristically high in Antarctic waters (Timmermans et al 2001) so that low nutrient to DOC release ratios should not limit use of krill-derived DOC by bacteria.

Table 3. Median and standard error of the mean (SE) for the carbon to nutrient and nitrogen to phosphorus ratios at each time step along the duration of the experiments (n = 8 experiments).

	C:N		N:P		C:P	
Time (mins)	median	SE	median	SE	median	SE
30	73.93	62.86	4.07	5.62	140.36	226.44
60	30.03	9.54	10.41	3.76	251.13	47.79
90	39.54	29.46	4.87	5.34	169.65	286.37
120	23.13	33.61	5.56	4.18	127.29	77.71
240	20.25	15.36	3.91	6.26	97.06	26.65

Krill was found to be an important source of recycled DOC in the system, supporting high rates of DOC release, comparable to those supported by phytoplankton (Table 1). Provided that submarine light availability at the time the study was conducted (January-February 2009) allowed photosynthetic rates to proceed over 14 hours whereas krill feed, and therefore release DOC, throughout 24 h (Morris et al. 1983). Whereas krill and phytoplankton release rates varied greatly, rates normalized to biomass, (g dry wt for krill and Chl *a* for phytoplankton) were relatively uniform, with normalized rates varying three and four fold, respectively. Phytoplankton DOC release rate estimates

include that released directly by phytoplankton as well as that mediated by protist grazing.

However, DOC release rates by zooplankton other than krill were not assessed, so that the release rates presented here should be considered minimum estimates of the total DOC released by zooplankton. The contribution of krill to DOC release is much greater than the average value of 12% of DOC production contributed by zooplankton calculated by Nagata (2000) for the global ocean. Even though the estimate derived here refers to krill alone and does not include the contribution from other components of the zooplankton community, such as copepods. Phytoplankton DOC release rates ranged from 40.43% to 72.65% of total organic carbon production, well above the range (3% to 47%) reported for the same region by Morán and Estrada (2002). Yet, the combined DOC production by phtytoplankton and krill was well above that of phytoplankton alone, averaging 205 ± 77.7 mmol C m^{-2} d^{-1}, of which krill contributed 73.03 ± 21% (calculated from Table 3). However, the contribution of krill as a source of DOC varied greatly, due to the patchy distribution of both krill and primary producers in the region, ranging from 98% to 10% of the combined (krill + phytoplankton) DOC release rate. Indeed, Morán et al. (2002) reported that bacterial carbon demand often exceeds the carbon supplied as DOC by phytoplankton in Antarctic coastal waters, and suggested that such carbon was supplied by terrestrial inputs (Morán et al. 2002). However, the results presented here indicate that krill is an important source of DOC in the Antarctic ecosystem hitherto unaccounted for.

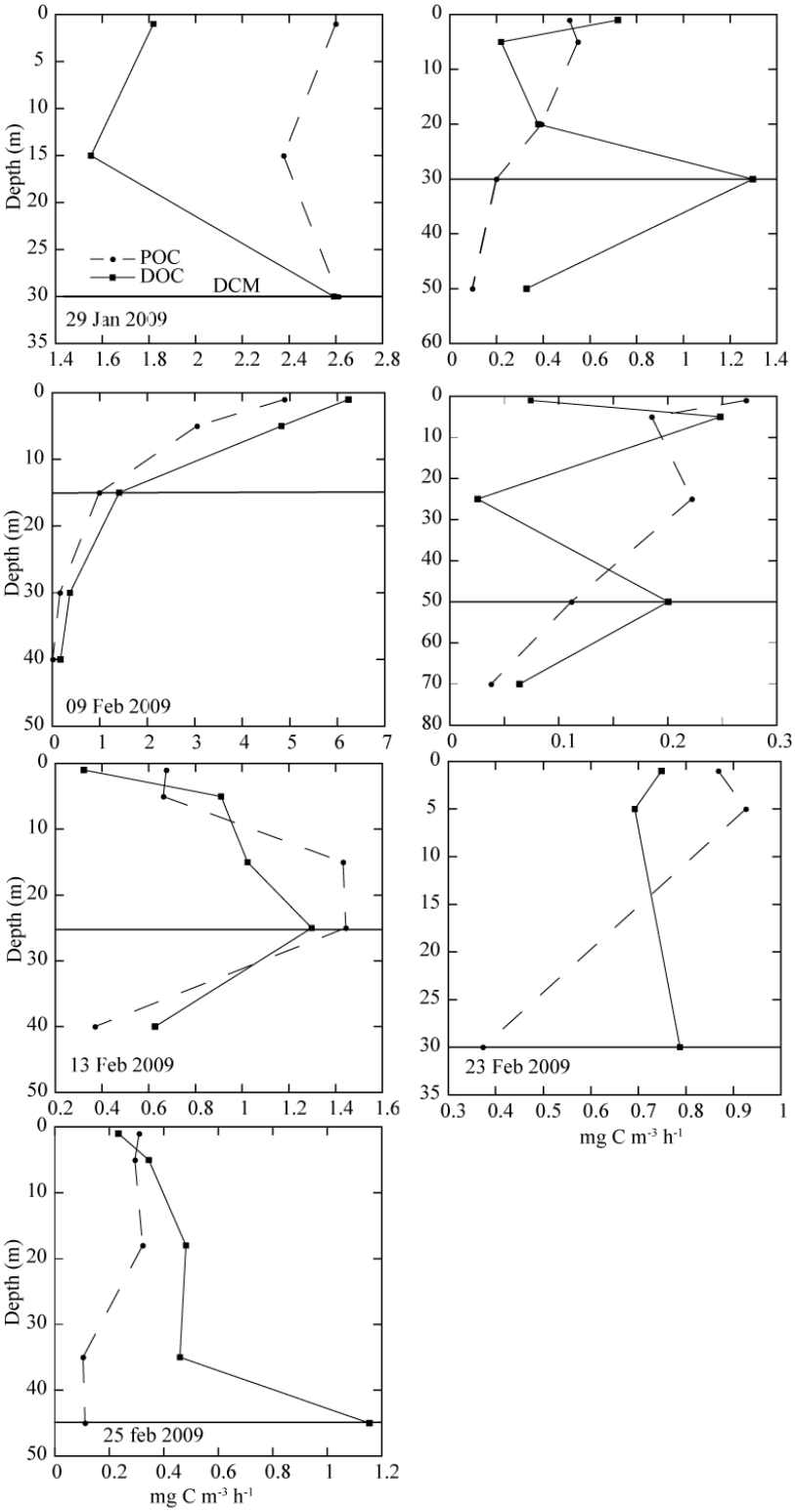

Figure 4. Vertical profiles of POC and DOC primary production. Horizontal line represents the DCM, always shallower than 50 m

The efficient recycling of nutrients by krill has lead to argue that these animals play a fundamental role in conditioning the Southern Ocean pelagic ecosystem to maintain it at a high productivity level (Smetacek. 2006;Tovar-Sanchez et al. 2007; Nicol et al. 2010). The results presented here suggest that krill not only recycle nutrients, to support phytoplankton growth, they do, in fact, retain nutrients differentially (Table 2) so that their contribution as an important source of DOC potentially used by bacteria, implying an important role of krill in the maintenance of microbial food webs, may be greater than anticipated. With gut cleareance rates of less than an hour, krill are expected to deliver DOC and nutrients at the initial rates and stoichiometry measured, resulting in a buildup of carbon relative to nitrogen, available to the microbial community. Moreover, whereas krill consume a broad range of prey, from phytoplankton to copepods (Hamner et al. 1983; Price et al. 1988), bacteria are too small to be removed by krill, except when associated to detritus ingested by krill. Hence, krill provides resources, DOC and nutrients, for bacteria without, unlike for phytoplankton, impinging severe losses on the bacterial community. Moreover, the characteristically patchy distribution of krill (Siegel 2000) suggests that DOC release by krill should play an important role as a source of patchiness in microbial activity in the region.

The importance of krill as a source of carbon for bacteria suggests that the recent observations of the decline in krill standing stock associated to reduced ice cover (Loeb et al. 1997; Atkinson et al. 2004) may have more pervasive effects in the Antarctic ecosystem than hitherto believed, affecting not only the metazoan food web directly, but the microbial food web as well. In addition to their potential effect on bacteria, the release of DOC by krill is sufficient in magnitude to play a significant role in carbon cycling in Antarctic waters. Indeed, the combined DOC release by phytoplankton and

krill, with a contribution of krill that matches or exceeds that of phytoplankton, indicates that the DOC pool, which is particularly small in Antarctic waters (Kähler et al. 1997), may turn over rather fast, at minimum rate of 10% ± 2% day^{-1} (calculated from Table 1). Hence, the decline of krill stocks may have effects on the remineralization of organic carbon. Based on the results presented here we, therefore, hypothesize that krill release products must greatly stimulate Antarctic bacterial communities with unforeseen consequences to the carbon balance of the Southern Ocean, a hypothesis that awaits experimental test.

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Annex - Antarctic krill as a source of dissolved organic carbon to the Antarctic ecosystem. Release of volatile and semivolatile OC products by *Eupahsia superba*

Methodology

The incubations were the same as the ones performed for DOC and nutrient measurements, but exchangeable dissolved organic carbon was only collected for the initial (time 0) and last (240 min) intervals. The measurement of EDOC followed the those of Dachs et al. (Dachs et al. 2005) and consisted in carefully siphoning 500 mL of water from the incubations onto an acid clean glass bottle.

Results and Discussion

The results presented in this appendix (table 1) were not included in the research article accepted in Limnology and Oceanography, as the data gathered spanned only 4 sites and final 4 hour incubations, so the decrease in release rates could not be calculated, and the dataset was insufficient to create an additional section in the article. However, the EDOC measured was sufficient to provide an estimate of daily release rates, and compare those values to the flux of CO_2 and exchangeable organic carbon for the same stations. The data shows a significant release of EDOC by Krill, comparable in magnitude to the air-water fluxes of OC and CO_2 (table 1). Indeed, Copepods contribute substantially to the particulate pool of dimethylsulfide (DMS) + dimethylsulfoniopropionate (DMSP) in coastal areas (Tang et al. 1999). It is then likely, that krill, as the major zooplanktonic grazer in Antarctic ecosystems, constitutes an important reservoir of particulate DMS in these waters. Previous investigations on

krill's effect on DMS release to Antarctic waters found enhanced release of DMS, and its precursor DMSP, in the presence of krill (Kasamatsu et al. 2004), providing indications that other volatile and semivolatile species may also be released by krill's feeding acticity. This release, can be attributed, in large part, to the "sloppy feeding" nature of Krill (Kasamatsu et al. 2004), but also to release via direct excretion or in fecal pellets (Kasamatsu 2008). Moreover, Krill ingest broken cells and in their guts they possess a powerful gastric mill (Kawaguchi et al. 2005) and enzymatic machinery (Saborowski and Buchholz 1999). This intense attack on ingested material is likely to degrade organic matter and produce a suite of volatile compounds that may be later released via excretion, as has been demonstrated for DOC, and quantified in this study. Thus, krill influences not only population dynamics, from the microbial loop, to the highest trophic level and the biogeochemistry of key elements (Tovar-Sanchez et al. 2007; Nicol et al. 2010), but can also, potentially, influence atmospheric chemistry and climate in a more direct manner than hitherto believed. As demonstrated by Kasamatsu et al. (2004) Krill influences regional DMS concentrations and its derivatives in the atmosphere and adjacent ice (Kasamatsu et al. 2004; Kawaguchi et al. 2005). Our results, pointing to a substantial amount of volatile and semivolatile organic compounds released by Krill, which can be potentially transferred to the atmosphere adds a further dimension to the already recognized central role of Krill in the Antarctic peninsula, since volatile compounds have a variety of effects in the atmosphere (Williams A 2004) , and may impact carbon dynamics at a larger scale than anticipated since atmospheric transport of krill products, a possibility, to date, never investigated, contributes to the redistribution of carbon in the ocean at a broader regional scale.

Table1. EDOC productin by Krill measured concurrently with the short time estimations of DOC release rates. Release were calculated as the difference between initial (time 0) and final (4 hours) EDOC concentrations in the formerly described incubations.

	krill biomass	**Krill Production**	**Air-water C exchange**	
Date	**g m^{-2}**	**μmol EDOC g^{-1} L^{-1} d^{-1}**	**mmol OC m^{-2} d^{-1}**	**mmol CO_2 m^{-2} d^{-1}**
09/02/2009	26.63	21.44	-10.22	-24.66
12/02/2009	56.70	63.03	19.45	-216.02
13/02/2009	19.52	437.18	16.69	-119.96
16/02/2009	36.41	74.03	-107.78	13.82
mean	34.81	148.92	-20.46	-86.71
s.e	9.32	111.72	34.48	59.43

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Williams A, J. 2004. Organic Trace Gases in the Atmosphere: An Overview.

Size-dependence of volatile and semi-volatile organic carbon content in phytoplankton cells

Abstract

The content of volatile and semivolatile organic compounds (VOC and SOC), was measured disrupting cells and quantifying the gaseous organic carbon released, measured as exchangeable dissolved organic carbon (EDOC) released in 9 phytoplanktonic species spanning 4 orders of magnitude in cell volume. EDOC content varied 4 orders of magnitude, from 0.0015 to 14.1 pg C $cell^{-1}$ in the species studied and increased linearly with increasing phytoplankton cell volume following the equation EDOC (pg C $cell^{-1}$)= $-2.44(CV)^{0.945}$ (cellvolume), with a slope very close to 1 indicating a constant increase in volatile carbon as the cell size of phytoplankton increased. The percentage of EDOC relative to total cellular carbon was small but varied 20 fold from 0.21 % to 4.71 %, and no obvious taxonomic pattern in the content of EDOC was appreciable for the species tested. The cell release rate of EDOC was difficult to measure, nonetheless, the results point to a potential flux of volatile and semivolatile phytoplankton-derived organic carbon to the atmosphere that has been largely underestimated and deserves further attention in the future.

Introduction

Recent methodological developments have enabled the measurement of the gaseous fraction of organic carbon dissolved in seawater, referred to as exchangeable dissolved organic carbon (EDOC). EDOC contains a numerous and largely unknown mixture of volatile and semivolatile organic carbon compounds (VOC and SOC). This bulk measurement has drawn the attention to this important, but largely overlooked, pool of carbon in marine ecosystems (cf. Dachs et al. 2005, Ruiz-Halpern et al. 2010). Macroalgae and microalgae contain numerous individual volatile and semivolatile organic compounds (Laturnus 2001, Colomb et al. 2008) and are considered to be important sources of these compounds to the ecosystem (Gschwend et al. 1985, Bravo-Linares et al. 2010, Ruiz-Halpern et al. 2010). Hence, release of VOC and SOC by marine autotrophs can support significant fluxes to the atmosphere (Sinha et al. 2007), with potentially important photochemical and climatic effects (Williams 2004). Indeed, some of the compounds released by phytoplankton are of global relevance, such as dimethyl sulfide (DMS) (Van Alstyne 2008). However, knowledge on VOC and SOC content of marine autotrophs is limited to specific compounds released by marine primary producers (organohalogens, isoprenes, alcohols, DMS…; (Giese et al. 1999, Sinha et al. 2007)) and the total pool of EDOC contained in marine primary producers has not been reported as such yet.

The organic carbon content of phytoplankton has been demonstrated in several studies to be closely scaled to cell size (e.g. Strathmann 1967, Verity et al. 1992, Menden-Deuer and Lessard 2000). Thus, allometric relationships have proven useful to calculate carbon-based biomass from estimates of phytoplankton size and abundance, as well as the carbon flow from phytoplankton of various cell sizes to consumers (Kiørboe

1993). However, these scaling laws are based on the assumed total pool since VOC and SOC were not considered in the particulate organic catbon (POC) values used, mostly derived from dried filtered samples (i.e., 'a minimum estimate of the total pool), as they did not include VOC and SOC nor, many times, dissolved organic carbon (DOC). Yet, reports of size-dependent release of DMS (Keller 1988) suggest that similar allometric relationships may exist for the cellular content of VOC and SOC in marine microautotrophs.

Here we report the EDOC content of phytoplankton cells and test its hypothesized size-dependence. We do so for nine phytoplankton species, from different taxonomic groups. We then searched for relationships describing the variability in EDOC content with phytoplankton cell size.

Materials and Methods

Estimates of cell size and EDOC concentration were derived for nine phytoplankton species (Table 1), available in cultures that spanned a broad size-spectrum. Additional species (e.g. *Chlorella marina*, *Navicula* sp. and *Heterocapsa* sp.) were tested but results could not be derived because the cells could not be lysed by the mechanical method used, and chemical methods can affect EDOC estimates (see below). The cultures were grown in triplicate 1L bottles under optimal temperature conditions at 18ºC for most species, 21ºC for *Synechococcus* sp. and *Prochlorococcus marina*. and 5ºC for *Melosira nummuloides*, and under continuous light conditions, in a nutrient-rich F/2 medium (Guillard and Ryther 1962), except for *Prochlorococcus marina*, which grew in Pro-99 medium (modified from Chisholm et al. 1992), and

Table 1. Cell volume, cell carbon content calculated using the equations from Verity et al. 1992, exchangeable cellular organic carbon content (EDOC), percentage of cellular C comprised by EDOC, percentage of cells ruptured by the mechanical procedure applied, and cellular carbon content derived from a compilation of previous studies that estimate carbon content from cell volume.

Species	Cell volume	C content	EDOC concentration	EDOC	disrupted cells	reported C	source
units	μm^3	pg C $cell^{-1}$	pg C $cell^{-1}$	% of cell C	% of total	pg C $cell^{-1}$	
Prochlorococcus marina (CCMP1375)	0.11	0.064	0.002	3.5	43.6 ± 5.6		
Synechococcus sp (CCMP833)	0.90	0.395	0.002	0.4	33.7 (n.a)	0.60	Verity et al. 1992
Micromonas pusilla (CCMP487)	11.0	3.4	0.03	1.0	31.7 ± 6.2	0.80	Montagnes et al 1994
Phaeodactylum tricornutum	42.8	11.1	0.11	1.0	68.2 ± 9.2	9.00	Strathmann 1967
Dunaliella sp	174	37.2	0.10	0.3	46.3 ± 6.8	41.7-52	Mullin et al. 1996
Phaeocystis sp	385	73.8	2.9	3.9	43.5 ± 16.6		Montagnes et al. 1994
Amphidinium carterae	1657	260	0.55	0.2	31.0 ± 3.8	95-259	Menden-deuer and Lessard. 2000
Melosira nummuloides (CCMP1903)	1954	300	14.1	4.7	20.8 ± 13.2		
Thalassiosira sp	2453	365	11.8	3.2	11.6 ± 4.2	316	Strathmann 1967

Melosira nummuloides, which grew in L1+Si medium following Guillard and Hargraves 1993). The duration of the experiments varied among the different species depending on population growth rates, as the measurements were performed at the onset of the stationary phase, when species reached their maximum expected cell abundance (data not shwown). These species were chosen because they encompass a wide variety of taxonomic lineages from diverse environments, and span several orders of magnitude in cell size.

Cultures were sampled daily or every two days to follow the changes in cell abundance. Changes in the abundance of cells for all species, except *Melosira nummuloides*, were quantified on fresh duplicated 1 ml samples counted in a FACSCalibur Flow Cytometer (Becton Dickinson). An aliquot of a calibrated solution of 1 μm diameter fluorescent beads (Polysciences Inc.) was added to the samples as an internal standard for the quantification of cell concentration. The red, green and orange fluorescence, and forward and side scattering signals of the cells and beads were used to detect different populations and to differentiate them from the fluorescent beads (Marie et al. 2000). Because *Melosira nummuloides* forms aggregates of cells, flow cytometry is not a reliable method to properly quantify cell abundance. Thus, changes in the abundance of cells were quantified by using 3.2-μL Fuchs-Rosenthal counting chambers (catalog No. 3720, Hausser Scientific, Horsham, PA) and counted under a microscope at 100 magnifications.

The cell volume of the different species was calculated by approximation to the nearest simple geometric shape (Hillebrand et al. 1999), from the dimensions of ca. 20 cells for each species measured at x 1000 under a Zeiss Axioplan 2 Imaging transmited light microscope. The cell diameter of *Prochlorococcus marina* cells was estimated in samples analyzed by scanning electron microscopy.

Extraction of EDOC followed immediately once the onset of the stationary phase was reached. Two replicate 400 mL acid-cleaned bottles were filled from each triplicate culture bottle. One was immediately processed for EDOC, and used as a blank to measure EDOC concentrations in the culture medium prior to lysing the cells. The second one received 17 g of 0.5 mm glass beads and 16 g of 0.1 mm glass beads, and was subject to intense vibration for 20 minutes on a high speed vortex to lyse the cells. The percentage of cells lysed (Table 1) was calculated as the ratio between the cell abundance measured before and after the vortexing procedure x 100. Immediately after vortexing the sample, EDOC was measured following the procedure described in Ruiz-Halpern et al. (2010). Briefly, each 400 mL bottle was bubbled with a stream of High purity N_2 gas, with a flow rate of 500-600 mL min^{-1}, for 8 minutes, determined to suffice to reach equilibrium. The gas evolved from the cultures, containing EDOC, was equilibrated in 50 mL of an acidified (H_3PO_4) ultra pure (free of carbon) water trap, to a pH of 2 to 3. This water was immediately transferred to 10 mL pre-combusted (4.5 h, 450ºC) glass ampoules and sealed under flame. EDOC was measured in duplicate on a Shimadzu TOC-Vcsh (total organic carbon) analyzer, with the sparge gas turned off.

The EDOC released by the lysed cells was calculated as the difference between EDOC in untreated *vs* lysed cultures. The EDOC content (pg C cell $^{-1}$) of each species was calculated as the ratio between the EDOC released (μmol C L^{-1}) and the number of cells lysed by the mechanical treatment, calculated by subtracting the number of remaining cells after the disruption process from the number of cells in initial conditions. The cell carbon content of the cultures was calculated using the relationship given in Verity et al. (1992), between cell volume (μm^3) and carbon mass (pg C $cell^{-1}$) with the form $C=0.433(CV)^{0.863}$

(Verity et al. 1992), where C is the cellular carbon content, and CV is the measured cell volume.

Results

The percentage of lysed cells in the phytoplankton cultures was 37 % ± 6 % S.E (calculated from Table 1). *Phaeodactylum* sp. cells were the most vulnerable (68% lysed cells), while *Thalassiosira* sp. had the lowest percentage of lysed cells (11 % lysed cells, Table 1). The carbon content derived from cell volume using the equations in Verity et al. (1992) were, for the most part, consistent with the values previously reported for the same phytoplankton species (Table 1).

The cellular EDOC content varied almost four orders of magnitude across species, from 0.0015 to 14.12 pg C $cell^{-1}$ (Table 1), and increased closely with cell size (Fig. 1), as described by the fitted model II regression equation (Sokal and Rohlf 1995, Legendre and Legendre 1998):

$\log_{10}$ EDOC (pg C $cell^{-1}$) = -2.44 + 0.945 (± 0.41) $\log_{10}$ cell volume (μm^3 $cell^{-1}$)

(R^2=0.86, p<0.05, n=9)

(Eq. 1)

Where the slope was not significantly different from 1 (t-test, P > 0.05), indicating that the EDOC concentration, per unit cell volume, was independent of cell size. EDOC represented, on average (± SE) 2.02 ± 0.67 % of the carbon content of phytoplankton cells (Table 1), but ranged greatly across species, from 0.21 % of the carbon content of *Amphidinium carterae*, the species with the lowest EDOC concentration to 4.71 % of the carbon content of *Melosira nummuloides*, the species with the highest EDOC concentration (Table 1).

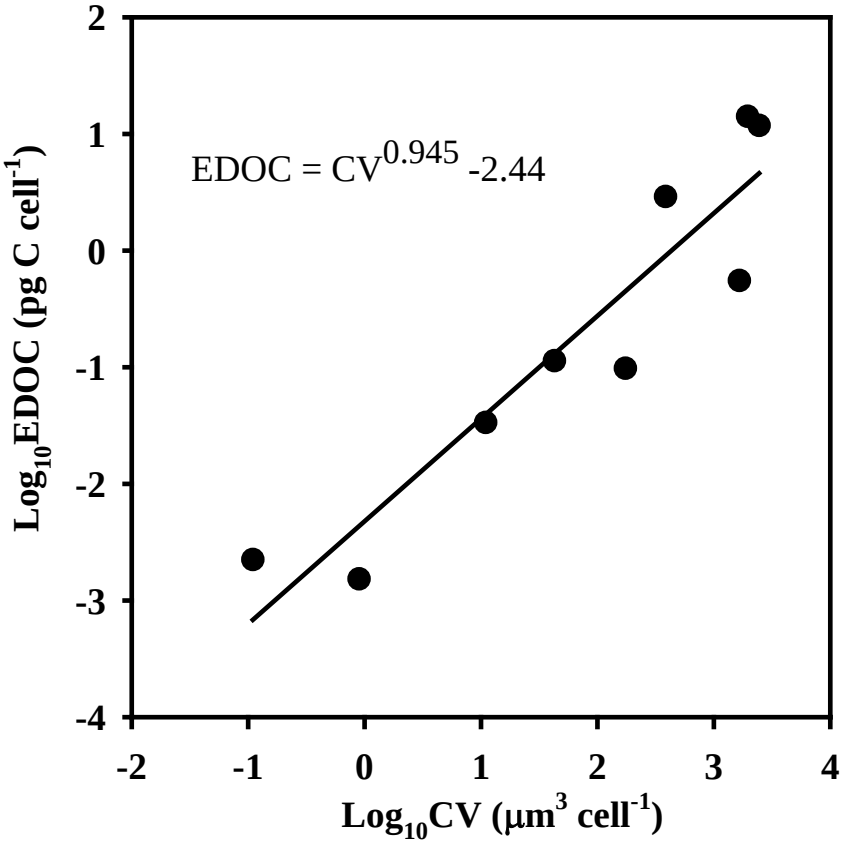

Figure 1. The relationship between the cellular volatile and semivolatile organic carbon content (EDOC) and the cell volume (CV) of phytoplankton species. The solid line shows the fitted model II regression line (R^2=0.86, p<0.05, n=9, Eq. 1).

Discussion

We provide the first estimate of the EDOC content of phytoplankton cell cultures, and assessment of the magnitude of such release in relation to body size and total carbon content of the cells. After careful consideration of the different options to disrupt the cell membranes of healthy cells (mechanical: vortexing, sonication, chemical: detergents such as sodium dodecyl sulfate (SDS)), mechanical disruption of the cells was chosen. Chemical treatments were deemed inadequate for 2 reasons: (1) they add carbon and as a foaming

surfactant could, potentially, contaminate our samples during EDOC extraction, and (2) are very aggressive denaturing agents, possibly affecting the volatility of some compounds. Sonication has previously been used to disrupt cells in yeasts (Borthwick et al. 2005), but the intense ultrasound waves at which the sample is subjected heats the sample and by cavitation, removes a significant amount of the gases dissolved from the medium (Butler et al. 1994), rendering this approach invalid for the purpose of measuring the EDOC pool after disruption of the cells. Vortexing the sample was the most appropriate method to obtain EDOC form the cells. However, there are at least two drawbacks that warrant consideration. Firstly, the turbulence created to disrupt the cells may facilitate the escape of some of the EDOC released during cell disruption, as a small headspace is left in the bottle, so that appropriate turbulence and cell-bead friction can be facilitated. Hence, EDOC measured should be considered as a minimum estimate of the total amount of EDOC released by the breaking of the cells. Secondly, it is not universal, and not all phytoplankton species are disrupted by this method. The small species (Chlorella sp.), as well as the two larger ones tested (Heterocapsa sp. and Navicula sp.) yielded virtually no cell breakage after the vortexing treatment, thus, no EDOC release could be calculated. The size differences between the species where vortexing failed, and the variability in the disruption percentage, suggests that other factors relative to the particular characteristics of each individual species affect the vortexing procedure in the disruption of the cells.

The results presented demonstrate that phytoplankton contain a small, yet quantifiable amount of EDOC that increases linearly with increasing cell size. The percent cellular C comprised by EDOC averaged 2.02 % and was independent of cell size, varying 20-fold across the species tested. Examination of the variability in the percent cellular C comprised by EDOC across species did not reveal any obvious phylogenetic pattern, as

similarly high % EDOC values (3.23 % to 4.71 %) were observed for a cyanobacteria (*Prochlorococcus marina*), a diatom (*Melosira nummuloides*) and a Prymnesiophyte (*Phaeocystis* sp., Table 1), whereas similarly low values (0.21 % to 0.31 %) were observed for a dinoflagellate (*Amphidinium carterae*), a chlorophyte (*Dunaliella* sp.), and a cyanobacteria (*Synechococcus* sp., Table 1).

Cell lysis, which is particularly important in the oligotrophic ocean (Agustí et al. 1998), leads to the release of the cellular contents of phytoplankton, delivering volatile and semi-volatile organic compounds. EDOC, which is likely to be composed of low molecular weight compounds, may also trespass cellular membranes, through gradient driven simple diffusion. Whereas the non-volatile forms of DOC will either be used by bacteria or accumulate in the water column (Ruiz-Halpern et al. 2011), the volatile and semi-volatile EDOC forms can also be ventilated, depending on the equilibrium concentrations between the atmosphere and the water surface. The few published reports suggest a prevalence of a net flux of total volatile and semi-volatile organic carbon from the atmosphere to the ocean (Dachs et al. 2005, Ruiz-Halpern et al. 2010). However, the ocean is a net source of some key volatile compounds, such as DMS, isoprene and halogenated compounds, to the atmosphere (Laturnus 2001, Sinha et al. 2007). Thus, a large portion of the EDOC measured in ambient water, or the atmosphere, may have a phytoplanktonic origin. Indeed, cell lysis is expected to be a source of volatile and semi-volatile compounds that are specifically produced by marine organisms, such as DMS or cyanobacterial toxins (Dembitsky et al. 2000), to the atmosphere, while the ocean will receive compounds which are produced by human activity (such as POPs) or specific of the terrestrial biome (e.g. terpenoids).

The results presented show that the cellular carbon comprised of volatile and semi-volatile forms increases proportionately with cell size, although the percentage of the cellular carbon comprised by these compounds vary greatly across species. The results presented contribute to our understanding of the sources of volatile and semi-volatile carbon in marine ecosystems. This knowledge would, when enhanced by additional estimates encompassing a broader taxonomic range that the one included in this first assessment, calculations of EDOC release rates with phytoplankton cell lysis or the assessment of how shifts in the composition and size structure of phytoplankton communities due to environmental factors may change the rates and stocks at which EDOC is produced and released into the environment, with potential large impacts to the ecosystem.

Our results suggest that changes in community structure may affect volatile and semi-volatile carbon release from phytoplankton. Indeed, release rates will depend both on community structure and cell lysis rates. These properties are not, however, independent, as some of the species with high volatile and semi-volatile carbon content, such as *Prochorococcus*, dominate the oligotrophic ocean where high phytoplankton lysis rates have been reported (Agustí et al. 1998, Agustí et al. 2001). Moreover, mortality rates of phototrophs increase with decreasing cell size (Marbà et al. 2007) suggesting also a major contribution to EDOC release when picophytoplankton communities dominate the water column. Picophytoplankton, and in particular, *Prochlorococcus* sp, are particularly sensitive to stressors such as high UV radiation (Llabrés and Agustí 2006) or pollutants (Echeveste et al. 2010) that induce high mortality on oceanic *Prochlorococcus* sp. and pico-phytoplankton. Moreover, future warming scenarios will most likely benefit the smaller

size spectrum of the phytoplankton communities such as *Prochorococcus* (Agawin et al. 2000, Moran et al. 2010) resulting in increased EDOC release in the water.

A scenario of a dominance of small phytoplankton species containing high fractions of volatile and semivolatile organic compounds, as *Prochlorococcus*, coupled with increased stress due to global change, may increase phytoplankton cell mortality and lysis rates, thus, resulting in increased EDOC release rates to the water column and, potentially, to the atmosphere. The published papers on DMS, VOCs and halogenated compounds, all of which contain carbon, show that phytoplankton release of volatile compounds, although not neglected, have been grossly underestimated, given the approach to identify and measure individual compounds, rather than bulk pools. Thus, the flux of volatile and semi-volatile organic carbon from phytoplankton may be greater than hitherto believed. Resolving the bulk cellular content of these compounds is a necessary step to assess the potential flux of phytoplankton-derived organic carbon to the surrounding waters, and even, the atmosphere.

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Potser avui m'he fet enfora

m'he perdut per la mar gran.

Mal que cridi i que gemegui,

sé segur que no em veuran.

Miquel Bauçà

(1940-2005)

Chapter 4

Annual benthic metabolism and organic carbon fluxes in a semi-enclosed Mediterranean bay dominated by *Caulerpa prolifera*.

Sergio Ruiz-Halpern, Raquel Vaquer-Sunyer and Carlos M. Duarte

Manuscript, 2011

Abstract

The incubations performed in the bay of Portocolom suggests *Caulerpa prolifera* to be a major contributor of Organic carbon (OC). While the ecosystem studied was in net metabolic balance year round, during the summer months, the community was net heterotrophic and community respiration (CR) was positively correlated to *C. prolifera* biomass, while NCP was negatively correlated. The benthic compartment represented, on average, 72.6 ± 5.2 % of CR and 86.8 ± 4.5 % of GPP. Dissolved organic carbon (DOC) production peaked in summer and was always positive, with dark incubations almost doubling the flux of incubations in the light. Exchangeable dissolved organic carbon (EDOC) oscillated between production and uptake, but was completely recycled within the system and remained around 10% of the DOC flux. The pools of bottom and surface DOC were quite high and dissolved CO_2 remained supersaturated with regards to the atmosphere. This suggests that, though in metabolic equilibrium, this ecosystem is able to export OC to adjacent areas but requires high allochtonous inpust during heterotrophic conditions likely derived from groundwater discharge and human activity in the watershed, delivered to the sediments through the high capacity of *C. prolifera* to remove particles from the water column.

Introduction

Coastal areas are especially active in the cycling of carbon on a global scale (Wollast 1998). Shallow waters enable the colonization of the seafloor by primary producers on a narrow band where enough light reaches the bottom (Gattuso et al. 2006; Markager and Sand-jensen 1992). These coastal areas cover only 10% of the ocean and

7% of the planet's surface, but are responsible for 20% and 10% of oceanic planetary production, respectively (Wollast 1998). Coastal areas are also responsible for a substantial portion of global carbon storage contributing to around half of the total burial of carbon in the oceans (Duarte et al. 2005, Kennedy et al. 2010). Furthermore, the autotrophic nature of these ecosystems implies that they export substantial amounts of carbon for consumption by heterotrophic communities elsewhere (Duarte and Cebrián 1996). Delivery of macrophyte carbon occurs mainly through three pathways (cf. Duarte and Cebrián 1996), direct herbivory by consumers, and subsequent incorporation to the foodweb (Hauxwell et al. 1998), shedding and fragmentation of detrital tissue (Kristensen et al. 1992), and direct release of dissolved organic carbon (DOC), which can account for 1-10% of the carbon fixed by macrophytes (Penhale and Smith 1977, Velimirov 1986, Barrón and Duarte 2009).

DOC is the fundamental pool of reactive carbon in the ocean, fueling the microbial loop in the oceans (Hansell and Carlson 2002). However, exchangeable dissolved organic carbon (EDOC), comprised of a wide variety of largely unresolved volatile and semivolatile organic compounds (VOC & SOC), is a largely ignored component of the DOC pool, which is operationally restricted to non-purgeable dissolved organic carbon in most applications (Spyres et al. 2000). However, the two studies thus far available (Dachs et al. 2005; Ruiz-Halpern et al. 2010) show that EDOC can be a significant component of marine carbon budgets. Furthermore, this carbon pool can be effectively exchanged with the air, affecting atmospheric chemistry (Arneth et al. 2010), or be transported and deposited elsewhere, resulting in the redistribution of carbon at regional and global scales (Dachs et al. 2005, Jurado et al. 2008). Macroalgae produce a variety of volatile and semivolatile compounds (Bravo-Linares et al. 2010), only a few of which are generally measured.

The single assessment available reports macroalgae to be an important source of EDOC to the ecosystem (Ruiz-Halpern et al. 2010). This study also demonstrates the sediments of the Greenlandic fjord studied to be an important source of EDOC, releasing an equivalent of 12% of POC deposited. However, whether these results are unique to the fjord studied or whether EDOC is an important component of carbon pools and fluxes in coastal ecosystems elsewhere remains unresolved.

Here we assess the metabolism of organic carbon fluxes of a semi-enclosed Mediterranean bay supporting a meadow of *Caulerpa prolifera*. In particular, we test whether the EDOC pool is a significant component of the carbon pools and fluxes in this ecosystem at the annual scale.

Materials and methods

The study was conducted in the human-influenced semi-enclosed bay of Portocolom in the Southeast of the island of Majorca (39º25´04´´N, 3º15´40´´E, Fig. 1). The study site was located on the Western area of the bay, sheltered from storms by land. The sampling area was covered by a dense meadow of the green algae *Caulerpa prolifera* growing on muddy sediments. The bay of Portocolom receives important loads of nutrients and organic matter through groundwater discharge (Basterretxea et al. 2010) as well as from deficient sewage systems. Fish farming activities, conducted in the interior of the Bay during 22 years until 2005, and the high nutrient and organic inputs from the surrounding town and boating activity have further increased the organic and nutrient pools in the sediments.

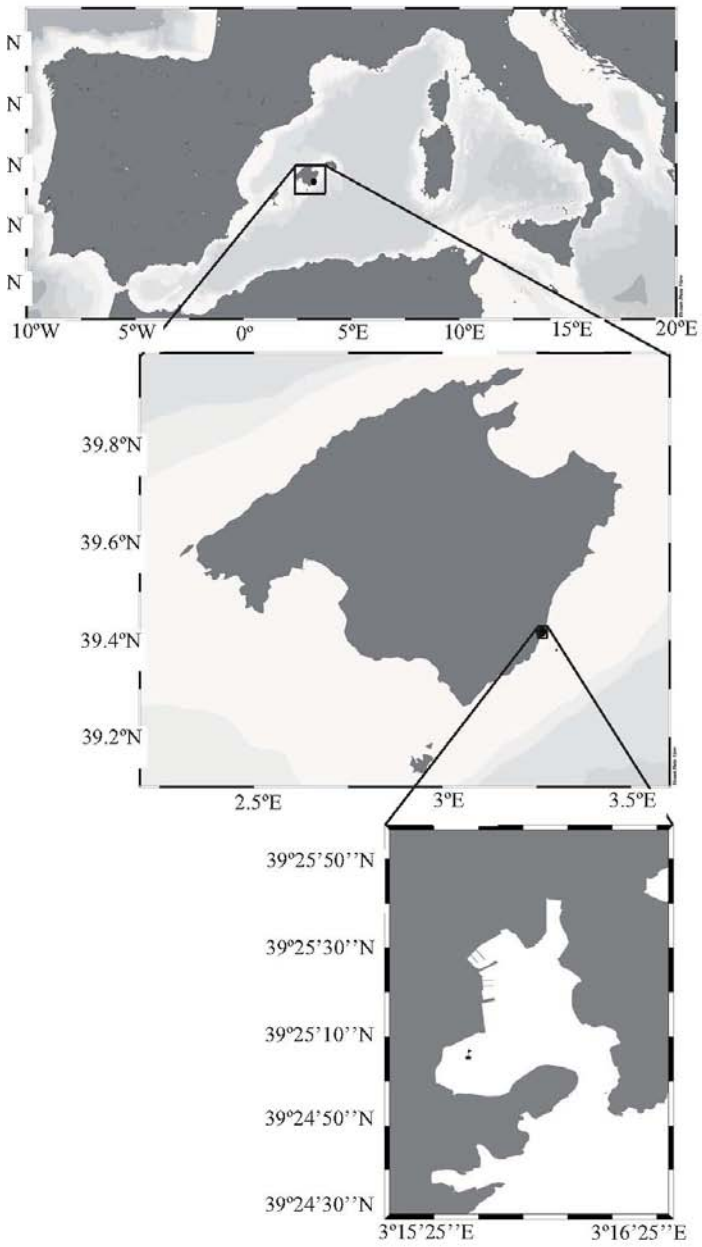

Figure 1. Map of the area showing the sampling station

Caulerpa prolifera (Forsskal) Lamouroux, 1809, is an opportunistic native species widely distributed throughout the Mediterranean Sea, except for the colder waters of the Gulf of Lyons and the Adriatic Sea (Sanchez Moyano et al. 2001), Sheltered and muddy sediments shallower than 20 m, a habitat abundant at the Bay

(Holmer et al. 2004), is the preferred habitat for growth of this macroalgae (Mateu-Vicens et al. 2010, Sanchez-Moyano et al. 2001).

The bay was sampled monthly from February 2009 to January 2010, and triplicate light and dark plexiglass sediment cores (0.005 m^2 i.d) were collected and incubated at in situ light and temperature conditions to assess benthic metabolic rates and carbon fluxes. Surface water samples were collected in the Bay using 20 L carboys, and sediment cores were extracted from 2.8 m depth by SCUBA diving, containing a random sample of the sediment community, dominated by *Caulerpa prolifera* and infaunal macroinvertebrates, as well as bacteria and phytoplankton associated with the sediments and living in the overlying water.

Metabolic rates of both the planktonic and benthic communities were derived through O_2 evolution in light and dark cores. Water samples were carefully siphoned into a variable number of 75-ml narrow-mouthed Winkler bottles. Between 22 and 26 bottles were filled for each experiment, resulting in 7–12 replicates per treatment, with most treatments having between 7/8 replicates. The samples were incubated in 'light' and 'dark' at *in situ* temperature for 24 h. Sediments cores were also incubated in 'light' and 'dark', but the incubation time of the sediment cores varied between 4 hours for dark cores and 24 h for light ones. The reason for performing shorter incubation times in the dark was due to high respiration rates that lead to anoxia in the dark cores if incubated for 24 hours. Before the incubation, rubber stoppers used to extract the sediment cores were replaced by plexiglass stoppers with an O-ring to avoid gas diffusion between water in the core and surrounding incubation water, and magnetic stirres inserted to ensure mixing along the height of the core. These stoppers had three sampling ports to sample water at the termination of the incubations. To determine initial oxygen content in the sediment cores, after changing the stoppers, Winkler

bottles were carefully filled by siphoning overlying water of the sediment cores using a silicon tube fitted in one of the three sampling ports. The same procedure was used to determine final oxygen content in the sediment cores after incubation. Initial samples were fixed immediately. All other bottles and sediment cores were incubated in 50 L tanks with a re-circulating, temperature controlled water bath with a light dimming mesh to mimic the conditions found in the field.

Dissolved oxygen was fixed immediately after the end of the incubation period and analyzed by high-precision Winkler titration, using a precise automated titration system with potentiometric (redox electrode) end-point detection (Mettler Toledo, DL28 titrator), after Carpenter (1966) and Oudot et al. (1988). Metabolic rates were calculated from the change in oxygen concentration after incubations, relative to the initial concentration and expressed as mmol O_2 m^{-2} d^{-1}. The rate of change in oxygen concentration in light and dark incubations provided estimates of net community production (NCP) and community respiration (R), respectively, and gross primary production is calculated as the difference between the NCP and R. The ratio between GPP and R provides an additional indication of the metabolic state of the community, as GPP/R < 1 indicate net heterotrophic communities while GPP/R > 1 correspond to autotrophic communities and ratios close to 1 indicate communities close to metabolic equilibrium.

The incubations also allowed estimation of the net production of DOC and EDOC by the community (Barrón and Duarte 2009). The initial concentration of DOC and EDOC was derived from samples taken from duplicate cores, collected in parallel to the onset of incubation to minimize differences with the initial conditions for these incubations. At the time of core collection, samples for measurements of DOC concentration in surface and bottom waters were collected, and water temperature and

the partial pressure of CO_2 measured. DOC was collected in duplicate 10 mL pre-combusted (4.5 h, 500ºC) glass ampoules sealed under flame and stored prior to analysis in the laboratory on a Shimadzu TOC-Vcsh with standard non-purgeable organic carbon (NPOC) techniques (Spyres et al. 2000). Standards provided by D. A. Hansell and W. Chen (University of Miami) of 2 and 44 µmol C L^{-1} were used to assess the accuracy of our DOC measurements. The overlying water from the sediment cores was carefully siphoned from the cylinder with a silicon tube and transferred to an acid clean bottle to measure the EDOC concentration following the procedure by Dachs et al. (2005). Water samples were bubbled with high grade (grade 5.0) pure nitrogen. The evolved gas containing EDOC, was redissolved in 50 mL of carbon free miliQ water acidified to a pH < 2 with concentrated (85%) H_3PO_4 and then transferred to triplicate pre-combusted (as for DOC) glass ampoules, stored in the laboratory and analyzed as for DOC, no field blanks were necessary, since production estimates were calculated as the difference between initial and final conditions, eliminating the need for blanks since any error form contamination was removed by the calculation procedure. Partial pressure of CO_2 was measured in air and water with a commercially available non dispersive infrared gas analyzer (IRGA, EGM4, PP Systems) at 1 min averages and an accuracy of ± 1 ppm. For water measurements, a peristaltic pump was used to circulate water through a gas exchange column (Mini-Module 1.25x9, membrane contactors, celgard). In the opposite direction a closed loop current of dry air was passed through the gas exchange column before entering the gas analyzer. Details on this method have been described in detail elsewhere (Silva et al. 2008). The IRGA was calibrated before each collection with two standard gases (pure N_2, no CO_2. And N_2 gas a partial pressure of CO_2 of 541 ppm (Calleja et al. 2005).

After the incubations were performed, biomass of *Caulerpa prolifera* from each core was estimated by gently separating the living tissue from the sediment and drying to constant weight at 60ºC in a drying oven. The biomass of *Caulerpa prolifera* was then transformed to carbon by assuming carbon to represent 25% dry weight (Duarte 1992). A 1 to 1 stoichiometric quotient was used to transform oxygen-based to carbon-based metabolic rates. The mass balance to calculate the allochtonous inputs of OC to the system was calculated following the equation:

0= GPP-CR-OC export + OC inputs (Eq. 1);

where OC export is the flux of EDOC+DOC measured in the incubations, the change in *C. prolifera* biomass was assumed to be included in the GPP, if there is a net increase in biomass or in CR if there is a decrease in biomass, this assumption may result in a slight underestimation of OC inputs since not all the biomass of *C. prolifera* has to be respired within the system (i.e herbivory by fish).

Results

Temperature in the bay Portocolom ranged from a minimum of 14 ºC in December to a maximum of 28 ºC in August and September, and CO_2 concentration in the water was supersaturated relative to the atmosphere throughout the study, with maximum values in midsummer, parallel to an increase in DOC concentration, both in surface and bottom waters (Table 1). A significant positive relationship ($p<0.05$) was found between temperature and dissolved CO_2 in the water and surface and bottom DOC, but this relationship was not seen for EDOC (figure 2).

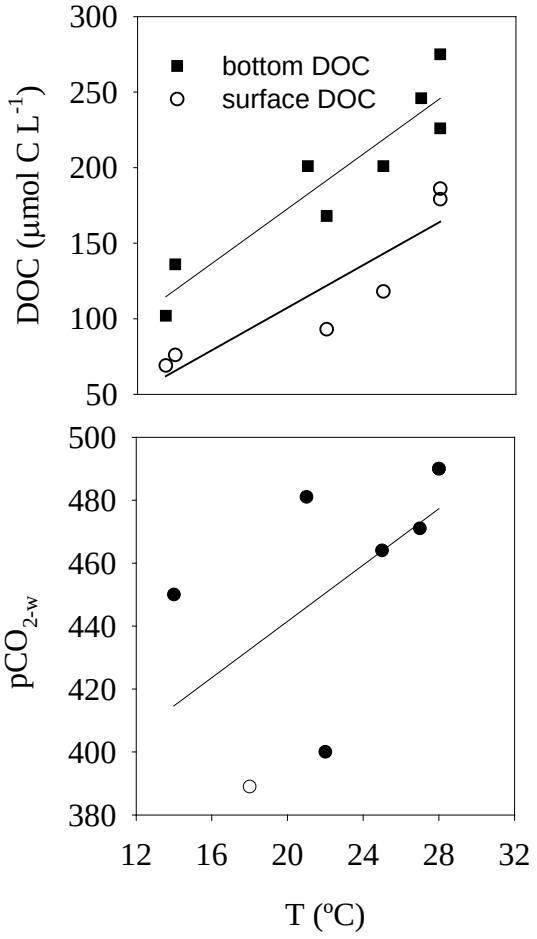

Figure 2. Relationships between bottom DOC (R^2=0.58), surface DOC(R^2=0.82), $pCO_{2\text{-}w}$ (R^2=0.34), with temperature. All relationships are statistically significant ($p<0.05$)

EDOC represented, on average, 22.4 ± 0.79 % of total organic carbon (TOC) (from table 1). Production of DOC and EDOC differed between light and dark conditions, with a higher production of both DOC and EDOC in cores incubated in the dark (Table 2, Figure 3). The EDOC production represented, on average, 10% of DOC production (from table 2). EDOC fluxes in the light showed both production and consumption (Figure 3), with a balanced annual flux (-0.1 ± 0.5 mol C m^{-2} a^{-1}, table 2). In dark conditions, however, there was a slightly positive, but variable, net flux of

EDOC of 1 ± 1.1 mol C m^{-2} a^{-1} (table 2). Net DOC fluxes in the dark were almost twice as high as those in the light (table 2), with an increase in net fluxes from winter to summer (Figure 3), yielding a positive net annual flux of 7.5 ± 4.1 mol C m^{-2} a^{-1} and 13.3 ± 5.6 mol C m^{-2} a^{-1}, respectively (Table 2, Figure 3). Biomass of *Caulerpa prolifera* increased over the spring and early summer but towards the fall and winter biomass did not return to values from the previous cold season, remaining relatively high. No significant differences were found between the biomass of dark and light cores (t test, $p>0.05$) (tables 2 & 3). Ecosystem (benthic + planktonic) metabolism was quite variable throughout the year, with the benthic compartment representing, on average, 72.6 ± 5.2 % of CR and 86.8 ± 4.5 % of GPP, contributing more to carbon fixation than the planktonic compartment. In addition, both community respiration and gross primary production increased in summer (table 3, Figure 4). Respiration increased more than GPP, resulting in net heterotrophic communities (NCP < 0) in the summer months. CR increased with increasing *C. prolifera* biomass (R^2 = 0.61 $p<0.05$, Figure 5), but an inverse relationship with NCP (figure 5 R^2=0.55 $P<0.05$ was found, GPP was not significantly correlated with *C. prolifera* biomass (Figure 5). However, the community remained in metabolic balance at the annual scale, with NCP = 0 ± 2.1 mol O_2 m^{-2} a^{-1} and a GPP/CR ratio of 1.0 ± 0.2 (table 3). There was no relationship between temperature or CO_2 and ecosystem metabolism and OC production.

Table 1. Sea surface temperature, partial pressure of CO_2 in water, EDOC in bottom waters, and DOC in bottom and surface waters in the bay of Portocolom.

Time		T	$pCO_{2\text{-}w}$	EDOC-bottom	DOC-bottom	DOC-surface
year	month	ºC	ppm	μmol C L^{-1}	μmol C L^{-1}	μmol C L^{-1}
2009	February	15.5	n.d	52	149	n.d
	March	16	n.d	16	134	n.d
	April	17	n.d	33	98	n.d
	May	18	389	35	n.d	n.d
	June	21	481	58	201	n.d
	July	27	471	45	246	n.d
	August	28	490	64	226	179
	Spetember	28	490	32	275	186
	October	25	464	97	201	118
	November	22	400	41	168	93
	December	14	450	78	136	76
2010	January	13.5	n.d	34	102	69
year total	mol m^{-2} a^{-1}±S.E	20.4±1.6	454±15	48.8±6.8	176±16.7	120±22.9

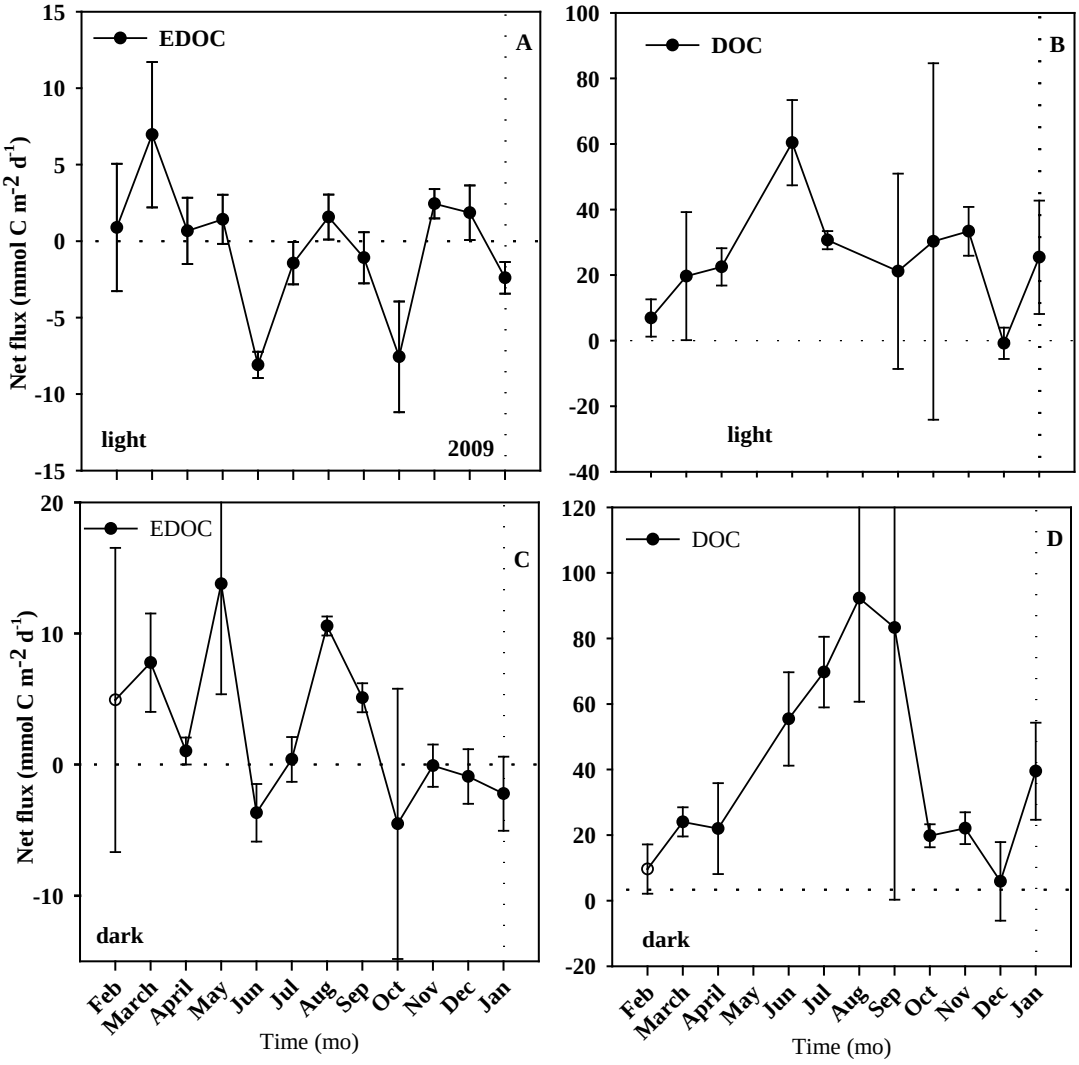

Figure 3. Net fluxes of EDOC and DOC from *C. prolifera* community over time. Mean ± SE.

Table 2. Net EDOC and DOC fluxes in light and dark incubations and biomass of *Caulerpa prolifera* in the bay of Portocolom. Means ± SE.

Light		EDOC		DOC		Combined OC		*C. prolifera*	
year	month	mmol C m^{-2} d^{-1}	S.E	mmol C m^{-2} d^{-1}	S.E	mmol C m^{-2} d^{-1}	S.E	g C m^{-2}	S.E
2009	February	0.9	2.9	6.9	4.0	7.8	1.4	17.3	2.1
	March	7.0	3.4	19.7	13.8	26.6	14.0	18.9	5.4
	April	0.7	1.5	22.5	4.0	23.2	5.4	73.3	9.1
	May	1.4	1.1	n.d	n.d	n.d	n.d	n.d	n.d
	June	-8.1	0.6	60.4	9.2	52.3	8.8	64.1	14.7
	July	-1.4	1.0	30.7	2.0	30.3	2.0	34.9	5.3
	August	1.6	1.0	n.d	n.d	n.d	n.d	40.5	5.0
	Spetember	-1.1	1.2	21.2	21.1	20.1	21.3	44.4	1.1
	October	-7.6	2.6	30.3	38.4	26.9	36.8	32.3	3.9
	November	2.4	0.7	33.4	5.3	35.8	4.9	n.d	n.d
	December	1.9	1.3	-0.8	3.4	1.1	3.8	35.9	1.9
2010	January	-2.4	0.7	25.4	12.2	23.0	11.9	35.9	1.9
year total	mol m^{-2} a^{-1}	-0.1	0.5	7.5	4.1	7.4	4.0	11.9	1.7

Dark year	month	EDOC mmol C m^{-2} d^{-1}	S.E	DOC mmol C m^{-2} d^{-1}	S.E	Combined OC mmol C m^{-2} d^{-1}	S.E	*C. prolifera* g C m^{-2}	S.E
	February	4.9	8.2	9.6	5.3	14.6	13.5	16.3	4.7
	March	7.8	2.7	24.0	3.1	11.9	1.0	15.5	6.7
	April	1.0	0.7	22.0	9.8	23.0	10.0	86.4	22.5
	May	13.8	6.0	n.d	n.d	n.d	n.d	n.d	n.d
	June	-3.7	1.6	55.5	10.1	51.8	11.5	62.0	10.4
2009	July	0.4	1.2	69.7	7.6	71.2	6.5	42.6	9.5
	August	10.6	0.5	92.3	22.4	102.9	22.7	36.9	8.0
	Spetember	5.1	0.8	83.3	58.7	88.4	58.0	41.0	17.9
	October	-4.5	7.3	19.8	2.5	19.9	6.7	44.6	7.9
	November	-0.1	1.1	22.1	3.4	22.0	2.9	n.d	n.d
	December	-0.9	1.5	5.9	8.5	5.0	7.0	67.4	10.3
2010	January	-2.2	2.0	39.5	10.5	37.3	10.1	67.4	10.3
year total	mol m^{-2} a^{-1}	1.0	1.1	13.3	5.6	13.4	5.6	14.4	3.2

The mass balance calculated using Eq 1. including the sources and sinks of OC measured, showed that the allocthonous carbon necessary to fuel respiration was, on average, in balance with the OC produced (available for export) in the incubations, in agreement with the overall metabolic balance calculated year round (Table 4).

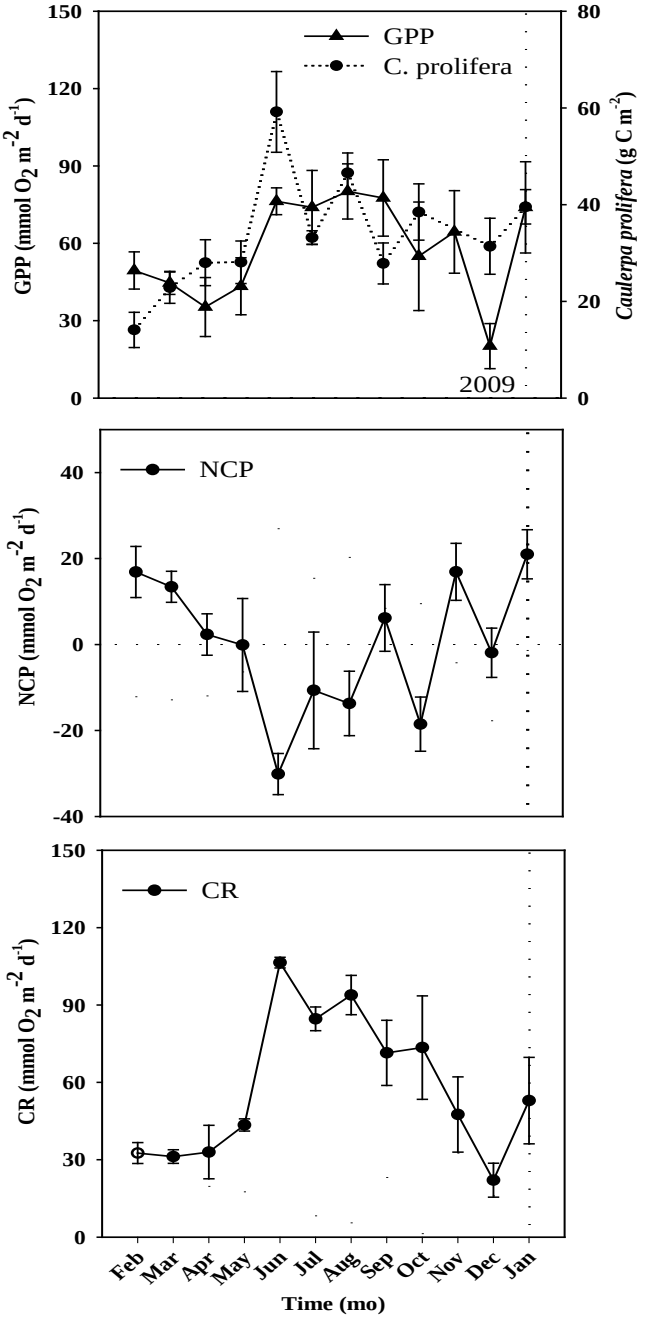

Figure 4. Temporal changes of community metabolism (GPP, CR and NCP) and C. prolifera biomass in Portocolom Bay. Mean ± SE.

Discussion.

The results presented demonstrate that the benthic community examined here contributes substantially to the TOC pool in the water column, both in a dissolved and exchangeable dissolved form. EDOC represents about a quarter of TOC, which is in agreement with the two available assessments (Dachs et al. 2005, Ruiz-Halpern et al. 2010) that report EDOC concentrations, and provides further evidence of EDOC as an important fraction of the TOC pool. DOC+EDOC production were highest during the summer months, the period with greater *C. prolifera* biomass, suggesting this macroalgae to be a major contributor to OC release. The net flux of DOC was always positive and exceeded GPP in the month of April. In contrast, EDOC fluxes oscillated from positive to negative across months, suggesting that EDOC may be completely recycled within the system. The yearly cycle of net DOC and EDOC fluxes in light conditions reveal a stronger dependence of net DOC fluxes on *C. prolifera* biomass than EDOC. However, OC fluxes were greatly enhanced in dark conditions, where photosynthesis is precluded and cannot be explained by enhanced production. Other mechanisms in the release of DOC+EDOC other than photosynthesis need be invoked and investigated further.

The net release of organic carbon from the community studied represents a source of export to adjacent ecosystems through advective and turbulent export and, in the case of EDOC, through the volatilization of these compounds to the atmosphere. In addition, DOC accumulated in the ecosystem from spring to summer, with an increase in DOC in bottom waters of 1.4 ± 0.74 μmol C L^{-1} d^{-1}, which is respired back to CO_2 in the fall and winter. The net flux of combined DOC is 7.5 ± 4.1 mol C m^{-2} a^{-1}, which roughly doubles the net amount of DOC produced by *P. oceanica* (Barrón and Duarte

Table 3. Community metabolism (NCP, CR and GPP), GPP/CR ratio and the biomass of C. prolifera over time in the bay of Portocolm. Means ± SE.

time		NCP		CR		GPP		GPP/CR		*C. prolifera*	
year	month	mmol O_2 m^{-2} d^{-1}	S.E	mmol O_2 m^{-2} d^{-1}	S.E	mmol O_2 m^{-2} d^{-1}	S.E		S.E	g C m^{-2}	S.E
2009	February	16.9	6.0	32.6	4.0	49.5	7.2	1.5	0.3	14.1	3.6
	March	13.4	3.6	31.2	2.6	44.7	4.5	1.4	0.2	22.8	3.2
	April	2.3	4.8	33.0	10.4	35.3	11.4	1.1	0.9	28.0	4.7
	May	-0.1	10.8	43.5	2.4	43.4	11.1	1.0	0.5	28.1	4.4
	June	-30.1	4.8	106.4	2.1	76.3	5.2	0.7	0.2	59.2	8.4
	July	-10.7	13.6	84.6	4.6	74.0	14.3	0.9	0.3	33.2	1.4
	August	-13.7	7.5	93.9	7.6	80.1	10.7	0.9	0.2	46.5	4.1
	Spetember	6.2	7.8	71.4	12.6	77.6	14.8	1.1	0.5	27.8	4.2
	October	-18.5	6.3	73.5	20.1	55.0	21.0	0.7	0.6	38.5	5.8
	November	16.9	6.6	47.5	14.6	64.4	16.0	1.4	0.9	n.d	n.d
	December	-1.9	5.7	22.1	6.6	20.1	8.7	0.9	0.7	31.4	5.8
2010	January	21.0	5.7	52.9	16.7	73.9	17.7	1.4	0.8	39.5	3.6
year total	mol m^{-2} a^{-1}	0.0	2.1	20.8	3.0	20.8	3.7	1.0	0.2	11.1	1.3

2009) and represents one third of the GPP measured at the site, rendering the *C. prolifera* community a greater source of organic carbon to the ecosystem than *P. oceanica*.

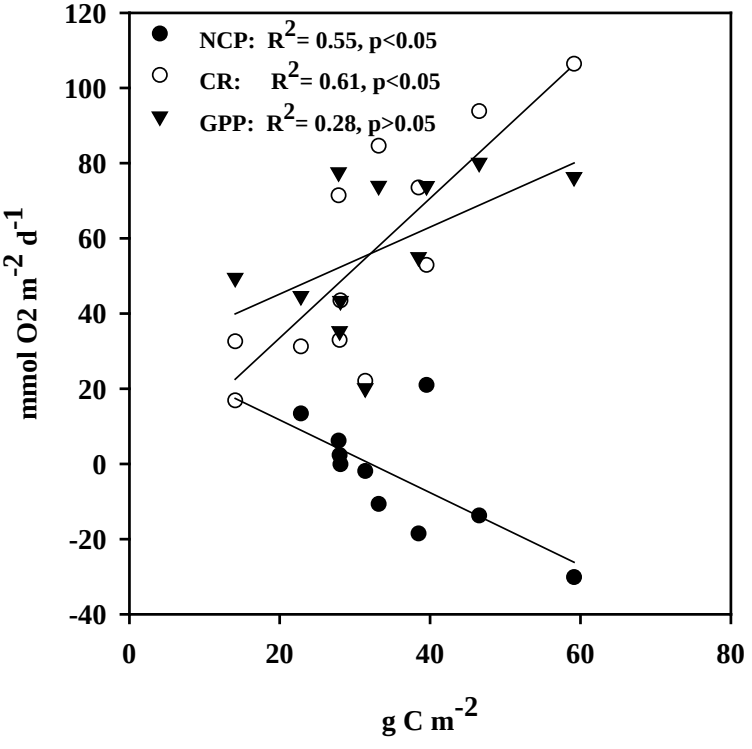

Figure 5. Linear relationship of metabolism (GPP, CR and NCP) with *Caulerpa prolifera* biomass

However, the release of organic carbon cannot be supported by community metabolism alone, since the community was in metabolic balance at the annual scale, with no excess carbon produced available to be exported. Hence, allochthonous organic carbon must be received by the community. Provided the community was a net source of DOC, these inputs could be derived from sedimenting particles, as demonstrated for a *Posidonia oceanica* meadow by Gacia et al. (2002). Indeed, Hendriks et al. (2010) experimentally demonstrated that *C. prolifera* were extremely effective in filtering out particles suspended in the water column, also explaining the high sulfate reduction rates associated with

sediments colonized by *C. prolifera* (Holmer et al. 2009). The metabolic deficit of the community averaged 35 mmol C m^{-2} d^{-1} but increased with increasing *C. prolifera* biomass, because respiratory processes are enhanced at a greater rate than photosynthetic ones with increasing *C. prolifera* biomass. Allochthonous inputs may also derive from groundwater discharge, which is high in this ecosystem (Basterretxea et al. 2010) and wastewater delivered by increased human population during the summer months, when the organic carbon deficit was largest, supporting the CO_2 supersaturation and high values of ambient DOC, both in surface and bottom waters. It is also possible that the complete recycling of EDOC within the system contributes to the accumulation of CO_2

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Table 4. Mass balance to calculate monthly inputs of allochthonous OC necessary to preserve the mass balance. The mass balance equation used was: 0=Allochtonous inputs+GPP-CR-OC export. Negative Allochtonous inputs indicate input of carbon from adjacent areas

year		GPP		CR		Combined OC		Allo. inputs	
	month	mmol O_2 m^{-2} d^{-1}	S.E	mmol O_2 m^{-2} d^{-1}	S.E	mmol C m^{-2} d^{-1}	S.E	mmol C m^{-2} d^{-1}	S.E
	feb	49.5	7.2	32.6	4.0	7.8	1.4	9.1	2.8
	mar	44.7	4.5	31.2	2.6	26.6	14.0	-13.2	5.0
	apr	35.3	11.4	33.0	10.4	23.2	5.4	-20.9	5.5
	may	43.4	11.1	43.5	2.4	n.d	n.d	n.d	n.d
	jun	76.3	5.2	106.4	2.1	52.3	8.8	-82.5	3.5
2009	jul	74.0	14.3	84.6	4.6	30.3	2.0	-41.0	5.1
	aug	80.1	10.7	93.9	7.6	n.d	n.d	n.d	n.d
	sep	77.6	14.8	71.4	12.6	20.1	21.3	-13.9	9.6
	oct	55.0	21.0	73.5	20.1	26.9	36.8	-45.4	15.6
	nov	64.4	16.0	47.5	14.6	35.8	4.9	-18.9	7.4
	dec	20.1	8.7	22.1	6.6	1.1	3.8	-3.0	3.9
2010	jan	73.9	17.7	52.9	16.7	23.0	11.9	-2.0	9.0
year total	mol m^{-2} a^{-1}	20.8	3.7	20.8	3.0	7.4	4.0	-7.0	2.0

In summary the data presented here show the *C. prolifera* community studied to be in metabolic equilibrium while supporting a high release of DOC to the environment, where it can be exported elsewhere. In addition these observations require high allochthonous carbon inputs likely derived from groundwater discharge and human activity in the watershed, delivered to the sediments through the high capacity of *C. prolifera* to remove particles from the water column. Furthermore, although EDOC represents roughly a quarter of TOC in the system, EDOC production represents only 10% of the DOC flux and is completely recycled within the system, suggesting an allochtonous input of EDOC, that could derive from air-water exchange (Dachs et al. 2005, Ruiz-Halpern et al. 2010), a pathway that has been demonstrated to constitute a significant means of entry for OC to marine ecosystems.

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Encara te sa clau des far on treballava,

Encara te sa clau d'ença que el jubilaren

…

…

Per falta de lluna, Venus se reflexa a la mar

Es ulls té ple d'estrelles de tant de cavil·lar

…

…

Es far de Ses Salines

Antònia Font

(2011)

Chapter 5

Multiscale cycles of exchangeable organic carbon and production by benthic communities in a coastal mediterranean site

Sergio Ruiz-Halpern Amanda Dorsett and Carlos M. Duarte

Manuscript, 2011

Abstract

The time series assessment of dissolved organic carbon (DOC), exchangeable dissolved organic carbon (EDOC) in the water and gaseous organic carbon (GOC) in the overlapping atmosphere, revealed a seasonal pattern with organic carbon increasing around spring and peaking in the summer time. Furthermore day night differences were also observed with increases in DOC of up to 25 µmol C L^{-1}, and 15 µmol C L^{-1} for EDOC. The interannual change in DOC and GOC over the five year period was at a rate of 5.7 % and 16.6 % respectively, but the ultimate causes of such increase cannot be elucidated here. EDOC amounts to 18% of total dissolved organic carbon representing an important fraction of the carbon budget in this system. Moreover the air-water exchange of organic carbon was almost always directed towards the water, concurrent with a net uptake of EDOC by the benthic compartment as measured by the bare sand and *Posidonia oceanica* meadow incubations performed. The dynamics of total dissolved organic carbon, at this coastal site, highlight the active nature of this pool of carbon and stresses the importance of atmospherically derived organic carbon for the biogeochemistry of this area.

Introduction

Coastal marine habitats are interface systems under the influence of terrestrial ecosystems and the open ocean. Coastal and open ocean ecosystems exchange materials through currents and upwellings (Pelegrí et al. 2005; Kosro et al. 1991), and terrestrial

environments influence marine ecosystems by the gravity-driven delivery of freshwater, nutrients and organic matter (Burnett et al 2006). However, lateral transfers are not the only pathway by which coastal marine ecosystems exchange materials, as air-sea exchanges can be particularly important in the coastal zone (Cai et al. 2006; Cai 2011).

Atmospheric deposition of particulate matter affects marine productivity (Duarte et al. 2006) and gas exchange between the air and water, modulated by windspeed, turbulence, surfactants and bubbles (Wanninkhof 1992) controls the partial pressures of numerous gases, both in water and in air, thereby determining the exchanges between these two compartments. For example, coastal ecosystems exchange significant amounts of CO_2, the major anthropogenic green house gas affecting global warming, with the atmosphere (Borges 2005). However, there are other significant organic gases, collectively known as volatile and semivolatile organic compounds (VOC and SOC), that are also subjected to exchanges between air and water (Dachs et al. 2005; Ruiz-Halpern et al. 2010). VOC and SOC are produced through natural processes, both on land and the ocean, and as a result of human activities (Jaward et al. 2004; Williams 2004). Coastal marine habitats are likely to support significant fluxes of VOC and SOC across the air-sea interface (Sauer 1981; Mantoura et al. 1982; McDonald et al. 1988). Inputs of VOC and SOC may derive from land or from other marine ecosystems.

Terrestrial plants emit a large number of these compounds, with an annual global release estimate of 1150 Tg of C (Guenther et al. 1995), which can be seasonally and spatially variable (Guenther 1997; Helmig et al. 2009). These terrestrial-derived compounds have been found in the water in coastal areas and even further offshore (Sauer 1981). Anthropogenic activities may also contribute to the VOC pool as one-third of total VOC emissions are from human origin (Guenther et al. 1995). Anthropogenic VOC

have also been detected in coastal sites, which tend to act as sinks for these trace gases (Mantoura et al. 1982). Marine organisms also produce VOC that can be released to the atmosphere (Sartin et al. 2001). The production of VOC has been documented for numerous marine primary producers, including phytoplankton (Chaptrer 3) and macroalgae (Laturnus 2000) and sediments have been reported to release VOCs (Ruiz-Halpern et al. 2010). Several VOC compounds have been detected in seagrass tissues (Kawasaki et al. 1998), although experiments evaluating the release of VOC by seagrasses are currently lacking. Guenther et al. (1995), calculated, on the basis of estimates based on a few VOC compounds,that the ocean emits 5 Tg C yr^{-1} of these VOC components to the atmosphere. Some of these ocean-derived VOCs may be photodegraded in the atmosphere or may re-enter the ocean elsewhere, rendering the atmosphere a pathway for the rapid transfer of VOC across distant marine ecosystems.

The cycling of VOC in coastal areas is likely to be complex and with potential profound effects on carbon cycling (e.g. Ruiz-Halpern et al. 2010). VOC can be removed from the water by processes other than volatilization, such as sorption onto particulate matter and photo- and biodegradation, especially in warm, biologically active areas (Wakeham et al. 1983). Coastal habitats are areas of intense biological activity, so that production and removal processes of VOC and SOC are likely to be enhanced. Marine primary producers (Laturnus et al. 2000; Sinha et al. 2007; Ruiz-Halpern et al. 2010) and the microbial community are significant sources of VOC (Kuzma et al. 1995) while bacteria can act as a sink (Wakeham et al. 1983).

Despite the potential importance of VOCs for carbon cycling and understanding of the mechanisms involved in the production of VOCs in coastal ecosystems (Kesselmeier and Staudt, 1999), estimates of VOC concentration and fluxes are largely limited to studies

assessing individual compounds (Williams 2004), with very few studies assessing total VOC fluxes (e.g. Ruiz-Halpern et al. 2010). Indeed, VOC is comprised of hundreds of individual compounds in the makeup of VOC (Goldstein and Galbally 2007), and traditional gas chromatography techniques for the measurement and quantification of single compounds are not able to resolve and quantify the total amount of VOC and SOC in water and air. The difficulties in resolving the total concentration of VOC accounts for the prevalence of individual-compound studies. This limitation was overcome by Dachs et al. (2005) through the development of an operational measurement of total VOC and SOC dissolved in the water, termed exchangeable dissolved organic carbon (EDOC) and the gaseous phase in the atmosphere, gaseous organic carbon (GOC) . This methodological advance has operationally resolved total VOC concentrations in water and air. EDOC has been estimated to comprise 30-40% of total dissolved organic carbon in the NE subtropical atlantic (Dachs et al. 2005) and the subarctic (Ruiz-Halpern et al. 2010). Moreover, this technique allows for the calculation of gross and net diffusive fluxes between air-and water, representing an advantage from previous attempts to measure total VOC in the atmosphere (Roberts et al. 1998). Here we use the approach developed by Dachs et al. (2005) to explore the daily variations, seasonal dynamics and interannual variability of DOC and EDOC in a Western Mediterranean coastal area and evaluate the gaseous-phase organic carbon present in the atmosphere. We also assess the production of EDOC in vegetated and unvegetated benthic habitats to test whether these communities act as sources or sinks of EDOC in the ecosystem studied.

Methods

A time series of DOC and EDOC in surface waters and GOC in the overlapping atmosphere, elapsing over five years was collected at the Faro de Salines coastal station, located in the Cabo de Salines. The Cabo de Salines lies in the Southernmost tip of the Island of Mallorca (39º16.0'N,003º3.0'E, Figure 1) in a pristine environment tens of kilometers away from the nearest populations. Data for DOC, EDOC and GOC was collected fortnightly from January 2006 to May 2011. Concurrently, sea surface temperature (SST) and wind speed were recorded using a callibrated, certified temperature probe and an Aandera metereological station . During the summer and fall of 2009, 2 diel cycles (August and October, 2009) of DOC and EDOC were obtained by sampling at 3 hour intervals.

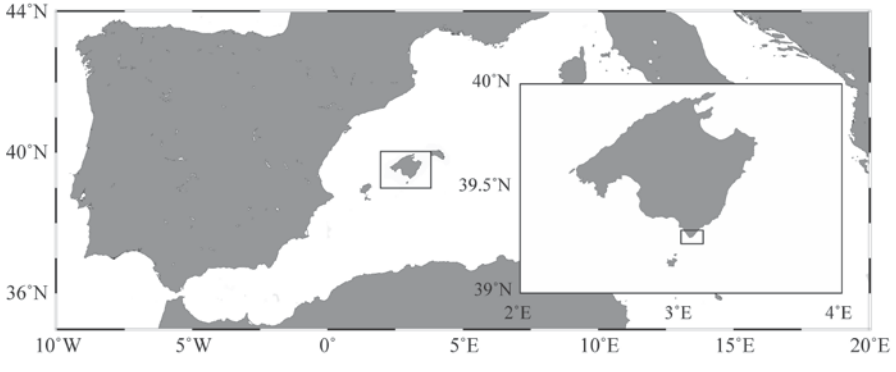

Figure 1. Map showing the location of Cabo de Salines in Mallorca Island, Spain (Western Mediterranean)

The coastal area around Mallorca Island supports a belt of a quasi-continuous meadows of the seagrass Posidonia oceanica, which has been shown to be a significant source of DOC (Barrón and Duarte 2009). We, therefore, tested whether the *Posidonia*

oceanica meadow is a significant source of EDOC. We did so through three separate experiments, conducted in July, August and October of 2009, respectively where bare sediments and seagrass meadows were enclosed in benthic chambers and incubated for 24 hrs.

Samples of seasurface water for DOC analyses were filtered and stored, acidified with concentrated H_3PO_4 to pH<2, in pre-muffled (5 hours 500ºC) 10 mL glass ampoules sealed under flame, until analysis on a Shimadzu TOC-Vcsh or TOC-5000 following standard non-purgeable organic carbon anlaysis (Spyres et al. 2000). EDOC and GOC were collected concurrently with DOC. EDOC samples followed the procedure described in Dachs et al. (2005) and Ruiz-Halpern et al. (2010) from aliquots of the same water sample collected for DOC analyses. GOC was also collected following Dachs et al. (2005) with the air pump placed close to the edge of the water in an area free of sources of contamination.

The incubations in the seagrass Posidonia ocenica and bare sediments were performed for 24 hours at a nearby site (4 m depth) using triplicated Polyvinylchloride (PVC) rings as described in Barrón et al (2006) and Silva et al. (2008). The PVC rings were inserted into bare sand or seagrass sediments by SCUBA divers. Trilaminate gas-tight bags were carefully attached to the PVC ring and fixed with rubber bands and cable ties to avoid leaks. Initial samples were taken from the surrounding water at the onset of the experiment. A sample port enabled extraction of water once the incubation was terminated. To obtain 1 L of water from the incubation chambers, an acid clean glass bottle fitted with a manual vacuum pump by means of an acid aged rubber stopper was connected to the sampling port with acid clean silicon tubing. Care was taken that the vacuum pump was connected downstream from the bottle to prevent sample water to get in contact with the pump and avoid possible contamination. The collection bottle was flushed with sample water

approximately 1.5 times the volume of the bottle and the first volume of water passing through the bottle and tubing, discarded, to avoid further contamination. The bottle with sample water was brought to the surface, and processed for EDOC as above. The volume of the chambers was calculated by the injection of a fluorescent dye and backcalculation of the volume from the dilution factor measured on a spectrophotometer. The flexible nature of the gas tight bag allows turbulence of the inner water with mixing times of around 5 s (Barrón et al 2006).

The flux of organic carbon in the incubations was calculated as the difference between the initial concentration of EDOC in the surrounding water and the final concentration after 24 h of incubation, expressed in mmol C m^{-2} d^{-1}. This measurement represents the net flux of the whole community, including the microbial and infaunal community living in the sediments, seagrass and associated epiphytes, and planktonic organisms trapped in the volume of water confined in the incubations.

Diffusive air–water exchange of OC was estimated using the difference between EDOC and GOC and the wind-speed dependence of the mass transfer velocity (k600) from instantaneous wind speeds from instantaneous wind speeds (U10, m s^{-1}) following the equation $k600 = 0.24U10^2 + 0.061U10$ (Nightingale et al. 2000). OC net diffusive fluxes (FOC) were estimated as the sum of gross volatilization ($FOC,VOL = k' \times EDOC$) and absorption ($FOC,AB = k' \times GOC\ H^{-1}$), where H^{-1} is the dimensionless Henry's law constant and k' is the gas transfer velocity for exchangeable OC estimated from $k600$ values and Schmidt numbers as in Dachs et al. (2005) and Ruiz-Halpern et al (2010).

Results

Water temperature during the study period ranged from 12 to 28.2 º C (Table 1). Wind speed was generally low for all seasons (on average, below 4 m s^{-1}), but slightly higher and more variable during the fall, concurrent with the end of summer transitioning to the winter and the stormy weather typical of the season (table 2).

Table 1. Seasonal means ± s.e for sea surface temperature DOC, EDOC and GOC. No significant differences were detected among seasons Tukey's post-hoc test ($p>0.05$)

season	T		DOC		EDOC		GOC H^{-1}	
	ºC	s.e	μmol C L^{-1}	s.e	μmol C L^{-1}	s.e	μmol C L^{-1}	s.e
winter	14.8	0.2	85.1	4.4	18.7	3.1	39.3	5.9
spring	20.0	0.7	85.8	3.9	16.9	2.3	29.7	4.7
summer	26.5	0.2	85.9	2.4	23.7	3.7	28.4	6.5
fall	20.2	0.8	92.1	3.8	25.4	3.5	23.5	5.8
total mean	20.5	0.8	84.7	1.8	18.2	1.5	28.3	2.9
range	12-28.2		39.9-118.2		1-70.2		0-100.7	
C.V (%)	24.20		16.70		70.20		65.80	

Temporal variability in DOC and EDOC

The time series of seasonal means showed a tendency towards higher values of OC during the summer and fall for both DOC and EDOC (Fig. 2). DOC averaged 84.7 ± 1.8 s.e along the study and was rather uniform among seasons, except for a tendency for higher concentrations in the fall (Table 1), when seagrass meadows release massive amounts of leaf litter that decomposes in the water column. EDOC and GOC were quite variable but differences among seasons were also not significant (table 1). DOC concentrations ranged from 39.9 µmol C L^{-1} in January 2007 to 118.2 µmol C L^{-1} in March 2011. EDOC and

GOC however, were more variable, with a minimum value of 1 and a maximum of 70.2 µmol C L^{-1} for EDOC, and below the detection limit and 100.70 µmol C $L^{-1}$for GOC. EDOC represented, on average 17.15 ± 1.33 % of DOC (range 1.06 - 33.87 %), obtained from all paired measurements of DOC and EDOC. The mean annual concentrations of DOC and GOC significantly increased over the 5 year period (Fig. 2), with DOC increasing by, on average, 5.7 % ± 3.5 s.e per year and GOC H'^{-1} increasing by 16.6% ± 12.02 s.e per year . However, EDOC concentrations did not show an increase over the studied period, and a decline in 2008 and 2009 was observed (fig 2).

The difference between EDOC and GOC indicates the direction and potential intensity of carbon exchange between air and water. GOC was higher than EDOC in 63 % of the sampling events, showing a prevalence of fluxes from air to water in this ecosystem, with a mean (± SE) difference of -8.9 ± 2.9 along the study (table 2), although some emissions were measured in the summer and fall of 2006, 2007 and 2008. On average, air-water exchange was directed towards the water in all seasons except for the summer where the water acts as a small source of EDOC to the atmosphere (Table 2). There were significant (ANOVA, $p < 0.02$) seasonal differences in these values, with GOC being higher than EDOC in winter, spring, and fall, indicative of high fluxes of VOC and SOC from the air to the water (Table 2). In contrast, the summer values were characterized by an overall equilibrium (mean ± SE difference 1.8 ± 3.4) between EDOC and GOC. Although the largest differences between air and water gaseous phase OC were in winter, air water-exchange is modulated by wind speed. Hence, the highest fluxes were recorded for the fall (table 2), where more variable and windier conditions prevail.

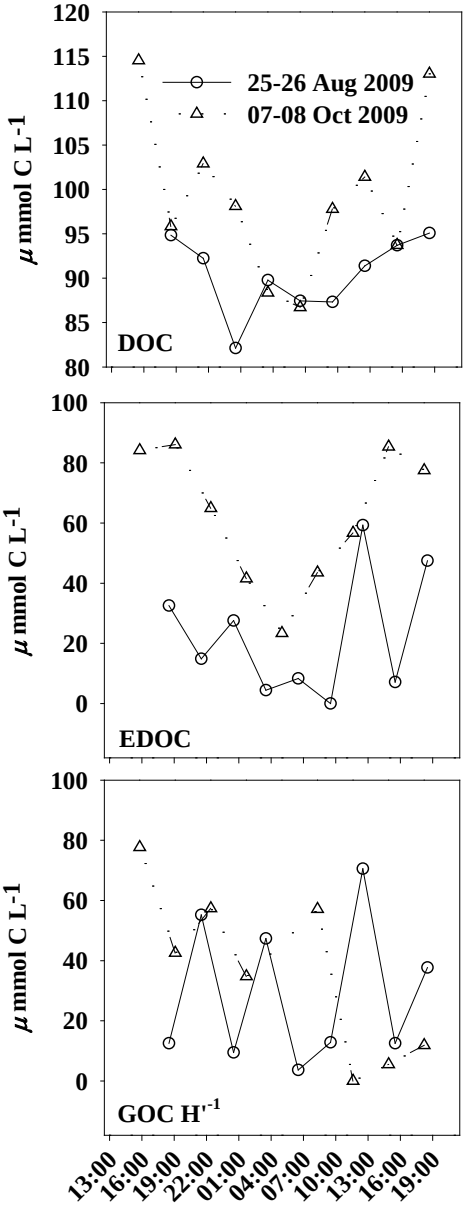

Figure 2. Temporal changes in mean (±s.e) annual, DOC, EDOC and GOC H'˙along the 6 year sampling period.

The diel cycles of ambient OC performed, showed a marked decrease in DOC and EDOC during the night time, with amaximum amplitude of 25µmol C L^{-1} in the case of DOC and 15 µmol C L^{-1} for EDOC (Figure 4). However, no clear pattern for GOC was observed (Figure 4). Despite the similarity in the daily cycles, October registered higher background concentrations of both EDOC and DOC, while GOC remained variable throughout the whole period.

Table 2. Season means ± s.e for windspeed, difference between EDOC and GOC (ΔOC), and and air-water exchange of organic carbon. Significant differences were only detected in ΔOC, Tukey's post-hoc test ($p<0.05$).

season	U		ΔOC(EDOC-GOC H'$^{-1}$)		OC flux	
	m s^{-1}	s.e	μmol C L^{-1}	s.e	mmol m^{-2} d^{-1}	s.e
winter	2.6	0.4	-23.6 (A)	6.0	-2.3	0.5
spring	3.6	0.3	-6.0 (AB)	4.5	-1.1	7.3
summer	3.8	0.4	1.8 (B)	3.4	0.5	2.5
fall	4.6	0.5	-4.4 (AB)	6.9	-3.3	4.7
total mean	3.7	0.25	-8.9	2.9	-2.1	2.3
range	1.0-9.4		(-93.7)-43.8		(-33.5)-12.1	
C.V (%)	54.7		28.3		34.9	

Benthic Incubations

The incubations performed showed that *P. oceanica* and bare sand support similar EDOC fluxes and showed a mean net uptake of EDOC for all months, except for bare sand in July. However, the fluxes were highly variable, especially in August where a net EDOC sink of - 6.5± 13.16 mmol C m^{-2} d^{-1} for *P. oceanica* and - 8.51±8.23 mmol C m^{-2} d^{-1} for bare sediments were recorded (Table 3), compared to an uptake of OC from the atmosphere of -13.08 mmol C m^{-2} d^{-1}. The exchange of OC between the air and water measured on the same dates as the incubations showed a net flux from the air to the water, consistent with

the net uptake of EDOC by the communities, although in July this flux was close to equilibrium (Table 3).

Discussion

The volatile fraction of the dissolved pool measured in this study represents about one fifth of the total (purgeable and non-purgeable) DOC pool, slightly lower, but in agreement with previous published reports in the subtropical Atlantic (Dachs et al. 2005), and the subarctic (Ruiz-Halpern et al. 2010). These observations provide, therefore, additional evidence that exchangeable OC is an important component of the carbon budget of the oceans and that the conventional measurement of DOC as non-purgeable DOC significantly underestimates DOC concentrations. Furthermore, our measurements highlight the variability of DOC and EDOC at different time scales, characterizing the seasonal variability of the dissolved pool of organic carbon in the Mediterranean coastal ecosystem investigated and that of the gaseous phase in the atmosphere, suggesting active processes controlling the concentration in both air and water.

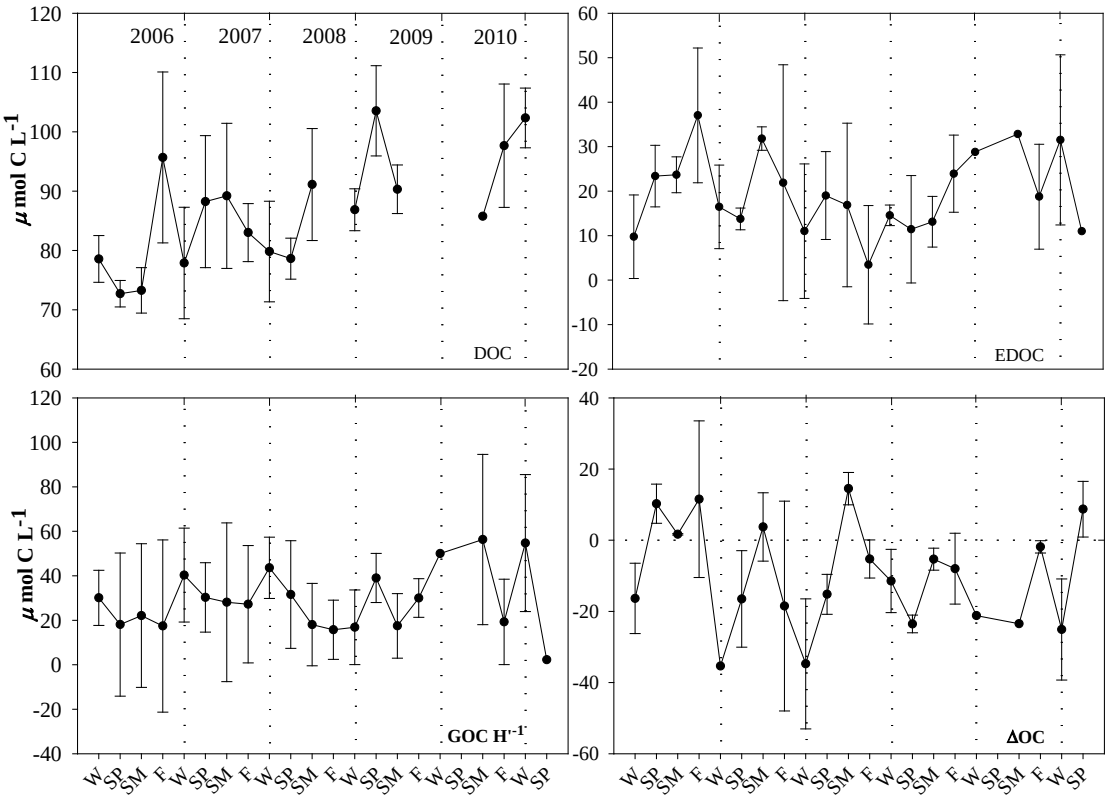

Figure 3. Seasonal cycles in mean (± s.e) DOC, EDOC, GOC H'$^{-1}$ and the difference between GOC H'$^{-1}$ and EDOC (ΔOC), from winter of 2006 to spring 2011. Some seasons could not be averaged for DOC due to missing data.

The seasonal DOC peaks measured in summer and fall, though not very marked are indicative of increased metabolic activity during the period of the year characterized by high irradiance and warm seawaters, in agreement with previous research documenting accumulation of DOC during the summer months (Williams 1995). Because the study area is devoid of any land sources of DOC, the seasonal increase in DOC is likely to derive from the release of the massive *Posidonia oceanica* meadow, which has been reported to support high release of DOC over spring and early summer (Barrón and Duarte 2009).

Table 3. Mean and s.d of EDOC flux for the incubations performed in summer and fall on benthic habitats close to the biological station and corresponding air-sea flux, measured on those days.

Inc. Type	*P. oceanica*		Bare sand		flux
Date	EDOC		EDOC		OC
	mmol C m^{-2} d^{-1}	s.e	mmol C m^{-2} d^{-1}	s.e	mmol m^{-2} d^{-1}
20-jul-09	-2.85	0.37	0.16	6.36	-0.84
26-ago-09	-6.52	13.16	-8.51	8.23	-13.08
08-oct-09	-4.37	2.03	-2.77	6.11	-43.31
mean	-4.58	3.38	-3.70	3.29	-19.08 ± 15.45

Global monitoring programs of several VOC species have determined a strong seasonal cycle for Ethane, Benzene, Acetylene, Toluene, Propane Xylene, (Helmig et al. 2009), Isoprene and other Terpenes (Guenther 1997), especially in the northern hemisphere. The seasonal cycle of GOC in our study is not as consistent as those derived from analyses of individual species, as GOC is composed of a mixture of VOC and SOC, which origin cannot be determined here, and may come from natural or anthropogenic sources on land, or from the ocean. Hence, the variability and characteristics of each particular compound may obscure the seasonal signal but there is still a tendency for GOC to peak in summer. Likewise the seasonal variation of EDOC is not very pronounced although a general agreement with GOC cycles is observed. Because, as pointed out by Dachs et al. (2005), EDOC and GOC are tightly coupled between the surface water and lower atmosphere, the difference between exchangeable OC in the water and that in air, which integrates both measurements, is where the seasonal signal arises more strongly, characterizing the direction of air-sea flux and constraining its magnitude. This difference showed a stronger potential flux towards the water during the winter time, and some efflux of OC in the summer and fall. While the production of land-derived VOC is expected to be greatest in the summer and fall, warmer temperatures favor higher Henry's law constants (H'),

displacing exchangeable OC towards the gaseous phase and increasing the partial pressure of VOC in seawater, thereby enhancing emissions. In contrast, H' are low at low temperatures, displacing this equilibrium towards the water phase (Staudinger and Roberts 2001), conducive to VOC uptake in the winter.

The flux experiments performed in *P. oceanica* and bare sediments point at these benthic communities as a sink for exchangeable organic carbon, with concurrent air-water flux of OC towards the water on those particular days. These experiments suggest that the driver for the prevalent ocean uptake of exchangeable OC from the atmosphere is due to rapid removal in the benthic ecosystem, sustaining a low partial pressure. EDOC uptake is likely to derive from microbial uptake, as microbial activity is enhanced in seagrass meadows (Jones et al. 2003). The net loss of EDOC in the benthic communities examined represents about 10% of the DOC typically supported by these ecosystems (Barrón and Duarte 2009). The diel cycles conducted provide further insights into the dynamic nature of EDOC in the ecosystem investigated. These cycles of OC revealed strong day night differences for OC dissolved in the water, but no apparent trend for GOC (Figure 4). This day-night differences are derived from a strong release of DOC and EDOC during the day time, while photosynthesis proceeds, but a removal of DOC and EDOC at night, possibly due to respiratory oxidation of these compounds. Whereas the net balance of DOC production has been shown to be positive for *Posidonia oceanica* meadows (Barrón and Duarte 2009), the net flux of EDOC over 24 hr was negative in all of our experiments, indicating that consumption prevailed over production, driving the uptake of atmospheric VOC that prevailed along the study.

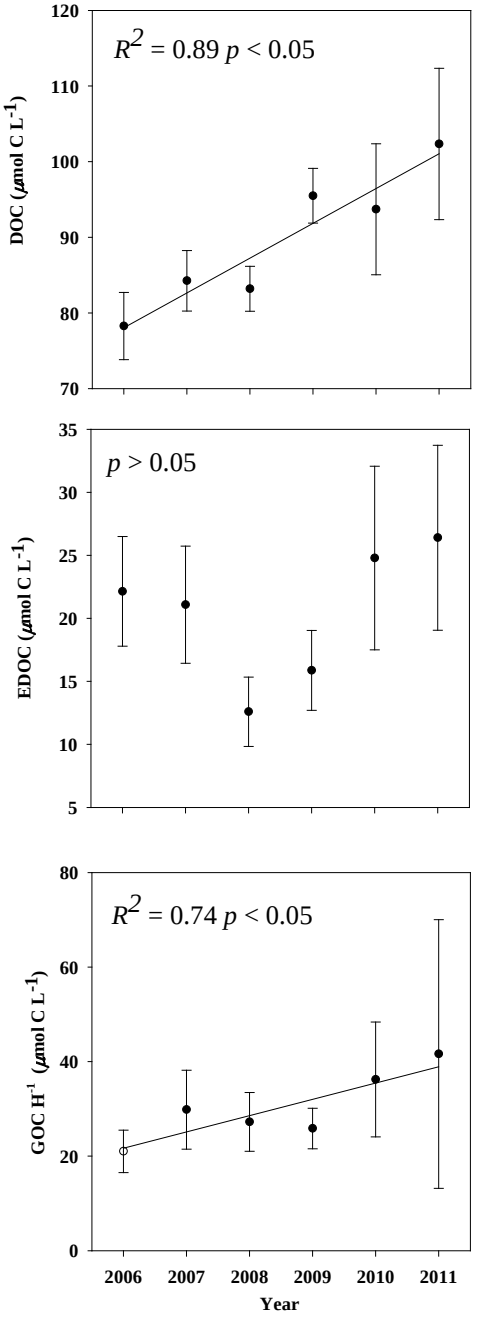

Figure 4. Variability along two 24 hour cycles of DOC, EDOC and GOC H'^{-1} in Cap Ses Salines (Open triangles 25-26 August 2009, open circles 07-08 October 2009).

The long-term trend towards an increase in DOC over the 5 year period at Cape de Salines is, to the best of our knowledge, the first documented interannual increase reported for the coastal ocean, but is in agreement with the well documented increase in DOC experienced in freshwater ecosystems due to a variety of factors, including warming and changes in atmospheric deposition chemistry (Evans et al. 2005; Monteith et al. 2007). However, we can only speculate on what the sources of the observed long-term increase in DOC in the ecosystem studied, away from any direct human influence, may be. However, a long-term increase in gaseous phase OC in the atmosphere was also observed. Whereas the vegetation in the region has not experienced any significant change, the sources for the increased OC may be distant, reflecting changes in the origin of the air mass or changes in either anthropogenic or land-derived emissions of VOC along the pathway of the air mass, where possible increases in human use for recreational purposes in the area may lead to increased emissions from exhaust fumes adding to the already enhanced emissions during the summer months.

In summary, the data presented here demonstrates, for the first time, that exchangeable OC is a highly dynamic biogeochemically active pool of carbon in coastal Mediterranean waters, with a nested variance structure determined by diel metabolic changes in the ecosystem, seasonal changes in ecosystem metabolism and water temperature and an unknown driver of interannual variability. Previous studies examining bulk EDOC and GOC pools and fluxes were either quasi-synoptic (Dachs et al. 2005) or involving a limited number of sampling events (Ruíz-Halpern et al. 2010). Also, the results presented here indicate that the coastal ecosystem studied acts as a sink for atmospheric VOC sustained by the net biological consumption of EDOC in the ecosystem. This finding suggests that atmospheric VOC should be important in ecosystem metabolic budgets and

that it must, therefore, subsidize the metabolic activity of heterotrophs in this ecosystem. Atmospheric VOC represents, therefore, a significant component of the carbon budget of the ecosystem studied, further supporting arguments that the atmosphere may be an important source of carbon for marine ecosystems both in the open ocean (Dachs et al. 2005, Jurado et al.) and in the coastal zone (Ruíz-Halpern et al. 2010).

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General Discussion

Within the framework of the complexities of the global carbon cycle in the ocean, this thesis aimed to contribute to the general knowledge of the biological and biogeochemical cycling of a neglected pool of carbon in most current accounts of carbon budgets, but becoming of increasing interest. We do this by providing data on the production of EDOC in different ecosystems and evaluating the oceanic and atmospheric stocks of gaseous phase OC, and its associated fluxes. Since primary producers are the major contributor to the DOM pool in the ocean, they were our primary focus, however, consumers and sediment processes are also recognized as key agents, not only contributing to the remineralization of OM, but also actively involved in the release to the water column.

These studies were carried out in regions of contrasting characteristics, including the coastal W Mediterranean, to investigate temporal dynamics, polar waters of the Subarctic seasonally, and Antarctica during summer. Besides the obvious differences in temperature and solar radiation input, due to their latitudinal range, these diverse ecosystems also present differences in their trophic status and stratification processes. Mediterranean stratification is mainly affected by increasing surface water temperatures towards the summer. In polar ice-margin waters, this stratification is primarily driven by ice-melting. Moreover, the W Mediterranean is a relatively oligotrophic environment (Lucea et al. 2005), whereas Antarctic waters are often classified as high nutrient low chlorophyll (HNLC) areas, but with occasional phytoplankton blooms recorded in spring and autumn (Chisholm and Morel 1991).

The specific objectives also differed across ecosystems. In the subarctic belt (Chapter 1) we looked at the seasonal dynamics of the air-water flux of exchangeable OC in relation to CO_2, and used a mass balance approach, although unable to completely constrain all inputs and outflows, to look at production/consumption processes of EDOC in the water column and searched for possible producers and consumers (macroalgae, sediments, and plankton community). After identifying macroscopic primary producers and sediments involved in the production of EDOC, experiments to elucidate production by microalgae were carried in the laboratory with phtytoplankton cultures, further expanding the search for internal sources of EDOC to the next trophic level, identifying Krill, a central node in Antarctic waters and a recognized agent in the release of DOC, as a source of EDOC (chapter 3).

In Chapter 2, we explore the spatial and temporal variability in summer EDOC and GOC concentrations and fluxes in the Antarctic Peninsula. The net air-sea flux of exchangeable organic carbon was compared to the flux of $CO_{2,}$ as this area is an important zone in the regulation of the earth's climate and is a recognized sink for this greenhouse active gas on a global scale (Sabine et al. 2004; Gruber et al. 2009). Chapters 4 & 5 were dedicated to examine the daily and annual cycles of EDOC and GOC in Mediterranean coastal ecosystems. Coastal areas are active sites for carbon processsing and seagrass and macroalgae have been identified as major sources of DOC to the environment (Carlson and Carlson 1984; Barrón and Duarte 2009). Thus, Chapter 4 focused on production processes of benthic communities over a year period, and its relationship to whole ecosystem metabolism, to determine the relevance of EDOC release to the carbon budget of benthic ecosystems. Chapter 5 explored variations in the concentration of EDOC, GOC and associated fluxes on several timescales, from diel to interannual.

More broadly, the research reported in this dissertation has been conducted under two main perspectives:

1. From a whole ecosystem perspective. The pools of oceanic EDOC and atmospheric GOC have been assessed, and the fluxes determined. Furthermore, the magnitude and importance of these stocks and processes have been placed in context of current knowledge on organic carbon content in the oceans (DOC) and carbon exchanges across the air-water boundary (CO_2)

2. From ecosystem compartments or single organisms, we have attempted to quantify the production or release of EDOC by several key components of the ecosystem, from macroalgae and sediments in the subarctic, to Krill in the Antarctic, or cultures of several phytoplankton species encompassing a wide size spectrum, to the characterization of whole benthic communities in the Mediterranean coast.

Overall, our results reveal a remarkably consistent pattern in the concentration of EDOC, GOC and its relationship to DOC. Indeed, the inventory of OC concentrations measured in the water and the atmosphere, across all ecosystems, showed the GOC pool to be

slightly higher than the EDOC pool (35.3±4.3 μmol C L^{-1} for EDOC and 42.9±4.5 μmol C L^{-1} for GOC) though equally variable, compared to a global mean of 82.9±1.2 μmol C L^{-1} of DOC, which are similar to the mean values found by Dachs et al. in (2005) for the Subtropical North Atlantic, suggesting EDOC and GOC to be rather uniform across ecosystems. However, EDOC and GOC values were, in general, more variable than DOC with concentrations ranging from non detectable to a maximum of 146 μmol C L^{-1} EDOC and 136 μmol C L^{-1} GOC among samples, but with 61 % and 65 % of the values in the 10 to 50 μmol C L^{-1} bracket, for EDOC and GOC, respectively . These global estimates imply EDOC to represent 29.1±1.5 % of total OC (DOC+EDOC).

Furthermore, this percentage was also quite similar across regions (Table 1), with higher values for Antarctic waters where, although the EDOC pool remained similar to other areas, the DOC pool in Antarctic waters was comparatively smaller (59.1 ± 3.6 μmol C L^{-1} DOC in the Antarctic peninsula, compared to 90.8±1.79 μmol C L^{-1} for the rest of areas combined), rendering the pool of EDOC proportionately more important than in other regions. Indeed, overall EDOC represents a mean 24.8±2.19 % of DOC across all ecosystems studied when the Southern Ocean dataset is removed, a value slightly smaller than that found in the subtropical North Atlantic by Dachs et al. 2005, driven by the low percentage of EDOC found in the Mediterranean. These values of EDOC in the water column, are significant, and render this fraction of the dissolved pool of organic carbon ubiquitous in the ocean, a quantitatively important pool of carbon that has been largely neglected to date.

In addition, the large spatial and temporal variability in EDOC concentration in all regions investigated, indicates that EDOC is a dynamic component of the carbon pool. Indeed, the fluxes of exchangeable organic carbon measured, tended, in almost all cases to exceed those of CO_2, with a slight but significant ($p<0.05$) prevalence of fluxes directed towards the water (59.7 ± 4.24 % of measurements), in agreement with the overall higher GOC values reported. Strikingly, the overall mean flux of exchangeable OC across all areas of -12.1±3.8 mmol C m^{-2} d^{-1}, was remarkably similar to, and not statistically different from, the overall flux of CO_2 (-10.9±11.3 mmol C m^{-2} d^{-1}, Table 1), albeit with great variability, with downward fluxes greater than 230 mmol C m^{-2} d^{-1} to net ventilations greater than 270 mmol C m^{-2} d^{-1}, while CO_2 maximum uptake by the oceans remained below 40 mmol C m^{-2} d^{-1} and below 30 mmol C m^{-2} d^{-1} release, with 67±4.81% of the values representing a source of CO_2 to the atmosphere in our dataset.

The dual source/sink nature of volatile and semivolatile organic carbon in the coastal ocean, as previously stated by Dachs et al. (2005) indicate that the air-sea boundary is an important pathway for organic carbon exchange that must also be included in ecosystem and global carbon budgets (Jurado et al. 2008), which only include CO_2 to date. Atmosphere transport is, therefore, an important conduit for the flux of organic carbon in the biosphere, connecting land and ocean and different oceanic regions across potentially vast distances, as atmospheric transport exceeds hundreds of kilometers per day. Additionally, the seasonal variability in the dynamics of exchangeable OC presented in chapter 5, together with the inherent variability in concentrations found across all ecosystems, shows that there is important spatial and temporal variability in atmosphere-ocean exchanges at different scales, and that environmental forcing plays a crucial role in the dynamics of exchangeable OC.

The EDOC production experiments conducted, both in the field and the laboratory, point to EDOC as an active component in the carbon cycle of the coastal ocean, representing around 10% of DOC production by marine primary producers. This value, however, needs to be taken with caution, as it is based on a yet a modest dataset. The results presented in chapters 1, 2, 4 and 5, provide further data to support the active role of biota in the biogeochemical cycling of EDOC in the water column, involving a wide array of organisms in different types of environments. Besides, the water column incubations from chapter 1 and benthic incubations from chapters 4 and 5, provided indications of consumption processes taking place in the water column or sediment, a research topic that requires further investigation, the consumers of EDOC have not been identified in this thesis. Heterotrophic bacteria, whether in the water column or in the sediments, are likely candidates as major contributors to the remineralization of EDOC, since they are the only significant consumer of organic matter in the ocean and are known to metabolize a number of individual VOC compounds, and have been recently reported to consume methanol in the Atlantic (Dixon et al. 2011).

Table 1. Mean (± S.E) DOC, EDOC, GOC H'$^{-1}$, the %EDOC contained in total dissolved OC (EDOC+DOC) and the air-water exchange of OC and CO_2. *Data from Dachs et al. 2005

	DOC		EDOC		(DOC+EDOC)		GOC H^{r-1}		Faw OC		FCO_2	
	µmol C L^{-1}	S.E	µmol C L^{-1}	S.E	%	S.E	µmol C L^{-1}	S.E	mmol m^{-2} d^{-1}	S.E	mmol m^{-2} d^{-1}	S.E
Subarctic	105.9	6.9	32.9	3.9	22.6	2.1	41.9	5.4	-1.6	1.7	-36.1	31.5
Mediterranean	86.0	2.0	18.8	1.7	17.8	1.5	27.9	2.9	-9.7	6.8	n.d	
Antarctic	59.1	3.6	43.1	3.0	41.8	2.7	45.5	3.0	-2.1	11.2	1.6	1.2
Atlantic*	80.6	3.3	46.3	17.6	34.0	7.1	56.3	19.2	-35.2	17.1	1.9	7.3
Total	82.9	1.2	35.3	4.3	29.1	1.5	42.9	4.5	-12.1	3.8	-10.9	11.3

An important area that could not be explored in this thesis is the abiotic forcing in the cycling of EDOC. The dissolution in the water of individual species contributing to EDOC and GOC is largely dependent on temperature, as it affects the vapor pressure and equilibrium concentrations, expressed by Henry's Law constant (H'). Hence, it is likely that the high values found in Antarctica, and to some degree in the subarctic belt, are not only the result of a comparatively small pool of DOC, but also a result of lower temperatures both in the water and in the atmosphere, that favor the equilibrium constants towards the water phase, displacing exchangable OC to the ocean. Additionally, UV radiation and OH radical formation greatly influence trace gases in the atmosphere and the water (Williams 2004), and the different radiation doses received in the different environments where our experiments were conducted have likely influenced the dynamics of exchangeable OC.

Implications for metabolic processes in the ocean

This thesis emphasizes the importance of the gaseous fluxes of atmospheric organic carbon to the ocean, and quantitatively recognizes the importance of this pool of carbon in the water. EDOC, largely consisting of an undefined mixture of volatile and semivolatile species, is likely to be rapidly metabolized, as they are small, low molecular weight molecules that are likely to conform a highly labile source of carbon, readily available to the heterotrophic microbial community. Indeed, Dixon et al. (2011) recently demonstrated rapid utilization of methanol as a carbon source for bacteria in the Atlantic. In chapter 4, benthic incubations showed EDOC to represent around 10% of DOC production; however, it is noteworthy that on an annual basis, EDOC production remained roughly in equilibrium, with production balanced by consumption. Furthermore, fluxes were dependent on the light environment, with incubations in the dark greatly enhancing the fluxes of both DOC and EDOC. In the absence of photosynthesis, other factors must contribute to these greater fluxes. These factors remain, however, to date unresolved.

The prevalence of a net uptake of exchangeable OC described in chapters 1, 3 and 5 points at a new pathway to provide the necessary subsidy of organic matter to account

for the deficit in carbon by heterotrophic areas in the world's oceans (del Giorgio and Duarte 2002). Furthermore, it is well established that atmospheric inputs enhance new production in the ocean (CO_2, Nitrogen and Iron inputs) (Duarte et al. 2006) and the fluxes measured here enhance the flux of organic carbon by a factor of 10 compared to previous estimates of dry and wet deposition of organic matter (Jurado et al. 2008), rendering exchangeable OC the main input of carbon through the atmosphere. These results provide further support to the idea that atmospheric exchanges with the ocean affect not only the autotrophic aspect of metabolism (by supplying CO_2 and nutrients), but also the other side of the balance, respiration, with potentially large impacts on the metabolic balance, affecting the capacity of the ocean as a sink for CO_2

In summary this thesis highlights the important role of volatile and semivolatile organic compounds, collectively measured as EDOC, in the coastal ocean, and calls for a need to expand the number of observations of the associated fluxes and an improved understanding of the biotic and environmental drivers of the cycling of EDOC in marine ecosystems.

Concluding remarks

During the completion of this thesis, several shortcomings were identified, that deserve further attention in the future and an effort to overcome those problems to advance in the characterization of exchangeable OC dynamics in the coupled atmosphere-ocean system. Firstly, uncertainties in the determination of EDOC and GOC, amplified by the inconsistency of field blanks calls for careful consideration and caution in the interpretation of the data. Since the efficiency is not 100%, one is tempted to believe our measurements are likely to be conservative and underestimates of true concentrations. However the problems detected with procedural blanks may be analogous to those found in the early stages of development of DOC measurements (Hansell and Carlson 2002), and the concentrations of VOC and SOC may be, in some cases, slightly overestimated. Efforts to improve blanks in other laboratories evaluating EDOC have been achieved at the expense of reduced recoveries. We, therefore, opted for obtaining more accurate but more imprecise estimates. Improving blanks without further losing recovery is, thus, a major requirement to improve current estimates. Lastly, the sampling method does not allow gathering high resolution data, temporally or spatially, and a fairly large volume of water, or a long period of time to pump

ambient air, is needed to obtain the data. This handicap limits the capacity to obtain estimates of EDOC and GOC at the scales possible for CO_2, for example, which can be measured underway in conventional applications. Addressing these issues would help further research on the role of volatile and semivolatile organic carbon in the oceanic carbon budget.

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Main Conclusions of the thesis

The main conclusions, ordered from more specific to more general, that arise from this dissertation are:

1. VOC and SOC are important components of the carbon budget of the subarctic fjord investigated. The results show that exchangeable DOC represents a large and dynamic carbon pool, accounting for about one-quarter of total OC in the water column. The atmosphere and benthic compartments acted as sources of EDOC to the water column, which could be consumed there. GOC and EDOC pools and fluxes at Kobbefjord are sufficiently large so as to be significant components of the carbon budget of this ecosystem.

2. We provide the first estimate of the EDOC content of phytoplankton cell cultures, and quantitative assessment of the magnitude of the potential release in relation to body size and total carbon content of the cells, as well as the release of EDOC by Antarctic Krill. The size dependence found in the release of EDOC by various phytoplankton species and Krill-mediated release of EDOC may have profound impacts on the carbon cycle at global scales, since the size spectrum and community structure of phytoplankton and the abundance of krill are varying under global change.

3. The Antarctic Peninsula is a highly dynamic area with particularly large spatial variability in EDOC pools, with low DOC concentrations in the southern ocean, the EDOC pool is comparatively more important here than in other areas. The close balance between uptake and ventilation of exchangeable OC in this area provides further support on the tight coupling and dual source/sink nature of coastal areas in the cycling of VOC and SOC.

4. EDOC production in Portocolom represents a small fraction of DOC production and primary producton and is generally completely recycled within the system. However, consumption of EDOC affects respiration rates, with potential effects on the metabolic status of ecosystems.

5. The multi-scale temporal variability of the dissolved pool of organic carbon in the Mediterranean coast and the gaseous phase in the atmosphere, suggests active processes controlling the concentration in both air and water. The fraction of EDOC measured represents a fifth of total DOC and is quite similar with previous studies in widely differing environments (subtropical Northeast Atlantic and Subarctic).

6. Exchangeable organic carbon is likely to be ubiquitous in the ocean, and highly reactive in nature, since it was present in all the environments sampled, albeit with large variability. Except for the Antarctic region, an area with especially low DOC, EDOC represents around 25 % of DOC.

7. The net air-sea flux of exchangeable organic carbon is a largely neglected pathway in the input and redistribution of OC in the oceans, which is comparable in magnitude to that of CO_2, and is likely to be the major component of allochthonous OC to the ocean.

8. A few key organisms in the production of EDOC have been identified, most of them primary producers (a wide size-spectrum of phytoplankton, macroalgae, marine macrophytes), but also consumers (Krill). All of them previously recognized agents in the release of DOC.

9. The high concentrations of bulk VOC and SOC reported in this dissertation are a hitherto unaccounted for important source of carbon for heterotrophs, with important consequences for the metabolic balance of the oceans

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