UNIVERSITAT POLITÈCNICA DE CATALUNYA BARCELONATECH

Effects of atmospheric deposition on microbial dynamics and composition in two anthropogenically-influenced contrasted coastal sites

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To my favourite parents and my favourite Pablos

"It always seems impossible until it is done"

(Nelson Mandela)

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Por último pero no por ello menos importante (como diría el gran filósofo del S. XXI, Mariano Rajoy, "o dicho de otra manera, igual de importante o más"), esta tesis también ha sido posible gracias a Pablo Tierz, mi compañero de viaje y mi mejor amigo. PT, tienes el don de ser una de las personas que alegra la vida de los que le rodean. Gracias por el tiempo compartido desde los hace ya más 12 años que nos conocemos, y especialmente los últimos 5 años y medio (5 años y medio ya, ¡qué pereza de relación!). Aunque nuestras tesis nos hayan separado físicamente, también nos han unido sentimentalmente. Como tú bien sabes una tesis doctoral conlleva mucho esfuerzo, estrés, y a veces frustración (también algún momento de alegría y satisfacción personal). Pero todos los problemas se hacen más soportables cuando tienes alguien con quién compartirlo, y sobre todo alguien con quién reírte de lo bueno y de lo malo (porque comparte tu mismo humor absurdo y un poquito macabro): "You can always make me smile". Entre experimento y muestreo, así como entre párrafos de cada capítulo de esta tesis, hemos disfrutado de cada momento juntos, en la parte del mundo que fuera, en la cercanía y en la lejanía. Gracias por estar siempre allí. Gracias por quererme y soportarme tal como soy. Y sobretodo, gracias por ser como eres. Personas como tú hacen que el mundo sea un poco menos feo, que no es poco.

Glossary of terms

List of the most common abbreviations and acronyms used in this thesis:

AIR: Aerosol induced ratio

HBA: Heterotrophic bacterial abundance

HBP: Heterotrophic bacterial production

Chl: Chlorophyll *a*

DIN: Dissolved inorganic nitrogen

DIP: Dissolved inorganic phosphorous

DOC: Dissolved organic carbon

DOM: Dissolved organic matter

HNF: Heterotrophic nanoflagellates

LNLC: low-nutrient low-chlorophyll

MS: Mediterranean Sea

Nano: Nanoeukaryoyes

Pico: Picoeukaryoyes

POC: Particulate organic carbon

Syn: Synechococcus

TIN: Total inorganic nitrogen

TIP: Total inorganic phosphorous

TOC: Total organic carbon

Abstract

The Mediterranean Sea is an oligotrophic basin, while the atmosphere above is affected by continuous emissions of anthropogenic aerosols and episodic Saharan dust events. These atmospheric inputs finally deposit (as wet or dry deposition) into surface waters, delivering high amounts of macronutrients and trace metals to surface waters. This process can constitute a main source of nutrient supply at certain times of the year, especially during the stratification period of the water column (May - October). In this thesis, we have assessed the effect of atmospheric particles on coastal planktonic communities following two approaches.

On one side, we have characterized the atmospheric deposition fluxes of the main macronutrients that limit or co-limit plankton growth and production in Mediterranean surface waters (i.e. inorganic nitrogen, phosphate, silicate, and organic carbon). To do so, we have measured the total atmospheric deposition (wet and dry) at two coastal locations of the northwestern Mediterranean with a contrasted anthropogenic footprint - Barcelona, urban location, and Blanes, with a lower degree of human impact, using passive collectors. We carried out a time-series of 4.5 years in Barcelona, and 3 years in Blanes. We observed that the deposition of the studied nutrients from the atmosphere occurs preferentially during the spring-summer season, coinciding with the stratification of the water column. In addition, we found some significant correlations between the nutrients released from the atmosphere and the concentration of chlorophyll and bacteria in seawater. In all, these results suggest that atmospheric deposition is an important source of new nutrients in coastal waters of the Mediterranean, with the potential to increase primary (autotrophic microorganisms) and secondary production (heterotrophic microorganisms) at certain times of the year.

On the other hand, with the aim to assess directly the effect of aerosols on microbial communities, we carried out microcosm experiments at the two coastal locations and in open waters of the western Mediterranean. We evaluated the effect of atmospheric particles from mineral (i.e. from the Saharan desert) and anthropogenic origin on marine phyto- and bacterioplankton at different times of the year. We found that aerosols did not produce significant effects on the microbial community during winter conditions, whereas atmospheric particles, especially from anthropogenic sources, significantly stimulated plankton growth and production during spring and summer. Anthropogenic aerosols enhanced bacterial metabolic processes significantly more than Saharan dust, what we mainly attribute to their higher content in soluble inorganic (mainly nitrogen, but also phosphate) and organic compounds. Furthermore, anthropogenic particles favored the growth of certain taxa of heterotrophic bacteria - mainly from the groups *Alphaproteobacteria* and *Bacteroidetes* - more than Saharan dust. Saharan dust instead enhanced preferentially the growth of cyanobacteria during summer. The overall effect of atmospheric particles on marine bacteria is dependent on the chemical composition of the aerosols, their solubility in the seawater, and the biogeochemical status of the seawater before the aerosol additions (deposition). Our results agree with others obtained so far in the Mediterranean, while we go one step further when assessing the role of anthropogenic aerosols on marine bacteria, a process that remains poorly studied.

Resumen

El Mar Mediterráneo es una cuenca oligotrófica cuya atmósfera se ve afectada por emisiones continuas de aerosoles antropogénicos, así como episodios de polvo sahariano. Estas partículas atmosféricas finalmente depositan en el mar (bien por vía húmeda o seca), aportando grandes cantidades de macro-nutrientes y metales traza a las aguas superficiales, pudiendo constituir su fuente principal en ciertas épocas del año, especialmente durante el período de estratificación de la columna de agua (Mayo - Octubre). En esta tesis, hemos evaluado el efecto de las partículas atmosféricas sobre las comunidades planctónicas costeras mediante la combinación de dos metodologías.

Por un lado, hemos caracterizado los flujos de deposición atmosférica de los principales nutrientes que pueden limitar el crecimiento y la producción del plancton en las aguas superficiales mediterráneas (nitrógeno inorgánico, fosfato, silicato y carbono orgánico). Para ello, hemos medido la deposición atmosférica total (húmeda y seca) mediante colectores pasivos en dos lugares costeros del noroeste del Mediterráneo con distinta huella antropogénica - Barcelona, sitio urbano, respecto a Blanes, menos influenciado por la actividad humana - durante 4.5 (Barcelona) y 3 (Blanes) años. La deposición de estos nutrientes desde la atmósfera ocurre preferentemente durante la época de primavera-verano, coincidiendo con la estratificación de la columna de agua. Además, encontramos algunas correlaciones significativas entre los nutrientes liberados desde la atmósfera y la concentración de clorofila y bacterias en el agua de mar. Estos resultados sugieren por tanto que la deposición atmosférica es una fuente importante de nuevos nutrientes en las aguas costeras del Mediterráneo, con la capacidad de incrementar la producción primaria (microorganismos autótrofos) y secundaria (microorganismos heterótrofos).

Por otro lado, con el objetivo de evaluar directamente el efecto de los aerosoles en las comunidades microbianas, realizamos experimentos de microcosmos en las dos localidades costeras y en aguas abiertas del Mediterráneo occidental. Evaluamos el efecto de las partículas atmosféricas tanto de origen mineral (es decir, procedentes del desierto del Sahara), como de origen antropogénico, sobre el fito- y bacterioplancton marino. Los estudios se llevaron a cabo en diferentes épocas del año. Observamos que los aerosoles no produjeron efectos significativos en la comunidad microbiana durante las condiciones de invierno, mientras que las partículas atmosféricas, especialmente las procedentes de fuentes antropogénicas, estimularon significativamente el crecimiento y la producción del plancton durante la primavera y el verano. Los aerosoles antropogénicos estimularon los procesos metabólicos heterotróficos significativamente más que el polvo sahariano, lo que atribuimos principalmente a su mayor contenido en compuestos inorgánicos (principalmente nitrógeno, pero también fosfato) y orgánicos solubles. Además, las partículas antropogénicas favorecieron el crecimiento de ciertos taxones de bacterias heterotróficas - pertenecientes principalmente a los grupos *Alphaproteobacteria* y *Bacteroidetes* - más que el polvo sahariano. El polvo sahariano, en cambio, aumentó preferentemente el crecimiento de las cianobacterias durante el verano. El efecto global de las partículas atmosféricas sobre las bacterias marinas depende de la composición química de los aerosoles, su solubilidad en el agua de mar, y el estado biogeoquímico del agua de mar antes de las adiciones (deposición) de aerosoles. Nuestros resultados coinciden generalmente con los obtenidos hasta ahora en el Mediterráneo, mientras que vamos un paso más allá al evaluar el impacto de los aerosoles antropogénicos en las bacterias marinas, proceso poco estudiado hasta la fecha.

Chapter 1. Introduction

1.1 The Mediterranean Sea

The Mediterranean Sea (MS) is a quasi-enclosed basin surrounded by land and connected with the Atlantic Ocean through the Strait of Gibraltar, with the Black Sea through the Turkish Straits (the Dardanelles, the Sea of Marmara, and the Bosphorus), and with the Red Sea through the Suez Canal (Fig. 1.1). With an area of 2.5 million km^2 and an average depth of 1500 m, it is one of the marginal seas more isolated from the ocean proper in comparison to its volume (Lionello et al., 2006).

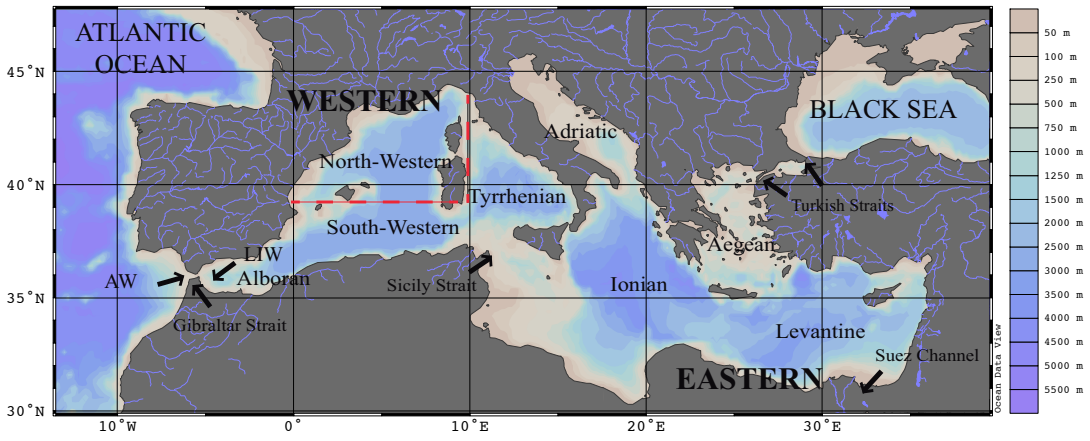

Figure 1.1. Map of the Mediterranean Sea with the bathymetry (colored legend on the right) and division of the sub-basins. The western basin is subdivided into the Alboran Sea, the North-Western MS, the South-Western MS, and the Tyrrhenian Sea; the eastern basin is subdivided into the Adriatic Sea, the Ionian Sea, the Aegean Sea, and the Levantine basin. AW: Atlantic Waters; LIW: Levantine Intermediate Waters. The North-Western region is delimited within red dotted lines.

The Mediterranean is divided in two sub-basins separated by the Strait of Sicily: the western and the eastern, that at the same time are sub-divided in different regions (Fig. 1.1). Thermohaline circulation inside the basin (Fig. 1.2) is driven by excess evaporation with respect to precipitation and river inputs, making the MS a concentration basin with an anti-estuarine circulation. Fresher surface Atlantic water enters through the Strait of Gibraltar and gets saltier and denser towards the east,

conforming the Levantine intermediate water (e.g., Bergamasco and Malanotte-Rizzoli, 2010; Estrada, 1996). As the unbalance between evaporation and precipitation plus runoff increases towards the east, the eastern basin is anti-estuarine respect to the western basin. Furthermore, water mass formation processes take place in the north-eastern and western basins, forming the Eastern Mediterranean deep water and the Western Mediterranean deep water (Bergamasco and Malanotte-Rizzoli, 2010; Fig. 1.2). At the basin scale, this thermohaline circulation results in a marked west-to-east gradient of decreasing nutrient (elements essential to life) concentrations, integrated chlorophyll and primary production in the epipelagic layer, and the export of particulate organic carbon (OC) from the surface layer (Krom et al. 1991; Moutin and Raimbault, 2002).

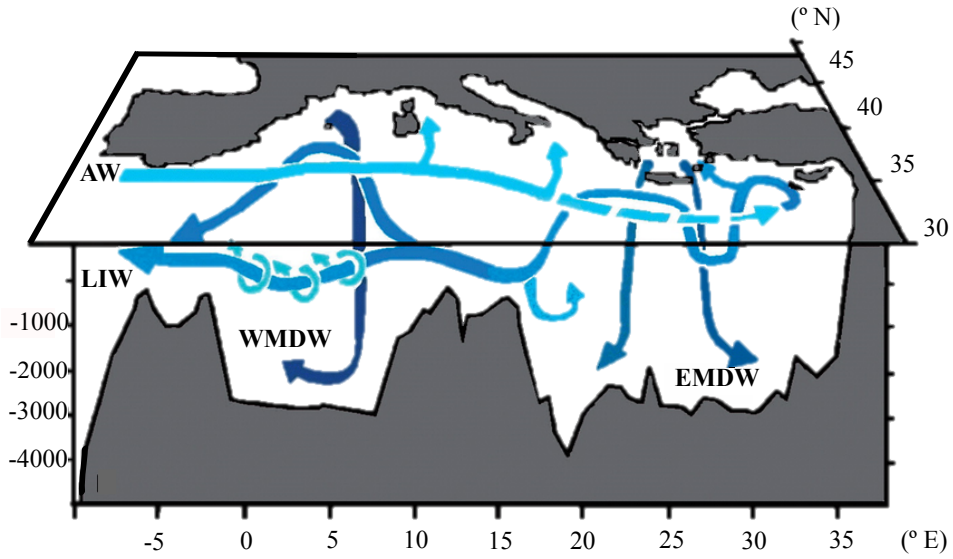

Figure 1.2. Scheme of the thermohaline circulation in the Mediterranean. Modified from Bergamasco and Malanotte-Rizzoli (2010) after Tsimplis et al. (2005). AW: Atlantic Waters; LIW: Levantine Intermediate Waters; EMDW: Eastern Mediterranean Deep waters; WMDW: Eastern Mediterranean Deep waters.

The MS is considered a low-nutrient low-chlorophyll (LNLC) region, containing some of the most oligotrophic waters in the world (Crise et al., 1999), although strong spatiotemporal gradients of nutrients and productivity can be observed within the basin.

The surface chlorophyll *a* annual average for the whole basin is 0.19 mg m^{-3}, and rarely exceeds 3 mg m^{-3} (Bosc et al. 2004; Santoleri et al. 2008; D'Ortenzio and Ribera D'Alcalà, 2009) (Fig. 1.3). The one-order-magnitude difference in chlorophyll *a* average between the western (0.26 mg m^{-3}; Santoleri et al. 2008) and the eastern (0.05 mg m^{-3}; Santoleri et al. 2008) basin has been attributed to the combination of the anti-estuarine circulation and biological processes related to the carbon pump (Crise et al., 1999), explained in the next section (Sect. 1.2, From the atmosphere to the ocean).

In the Mediterranean, as in most mid-latitude oceans, the summer period is characterized by the presence of a stable thermocline that separates the mixed layer (ML) – with high light intensity but depleted in inorganic nutrients – from deep waters richer in nutrients. During late autumn and winter, wind-driven turbulence and convective mixing produce the break of the thermocline and the ML extents, facilitating the entrainment of nutrients from bottom waters to surface waters and making such nutrients available to the surface ocean planktonic communities. Albeit the ML remains at depth < 30 m for most of the year (April – October; Fig. 1.4) in the whole basin, it can reach depths > 100 m at some points during winter and early spring. The increase in solar radiation at late winter – early spring yields a bloom of phytoplankton, only conspicuous in the northwestern (NW) area (Siokou-Frangou et al., 2010). Succession of major phytoplankton groups takes place in parallel to this physico-chemical seasonal variability, as established by Margalef (1997). During the bloom, large diatoms tend to dominate the phytoplankton community. Then a succession is observed toward smaller cells, picophytoplankton (< 2 μm) and cyanobacteria, accounting for a higher percentage during the most oligotrophic summer season (Gutiérrez-Rodríguez et al., 2010, Marty et al., 2002). A certain interannual variability is observed and correlated with the variability of winter convection intensity, though (Herrmann et al., 2013).

Apart from the seasonal bloom, other mechanisms may enhance fertility at certain times of the year, as the supply of external sources of nutrients from river runoff or from the atmosphere (Durrieu de Madron et al., 2011; Estrada et al., 1996). Coastal areas are especially affected by these sources, showing the highest chlorophyll concentrations (Fig. 1.3).

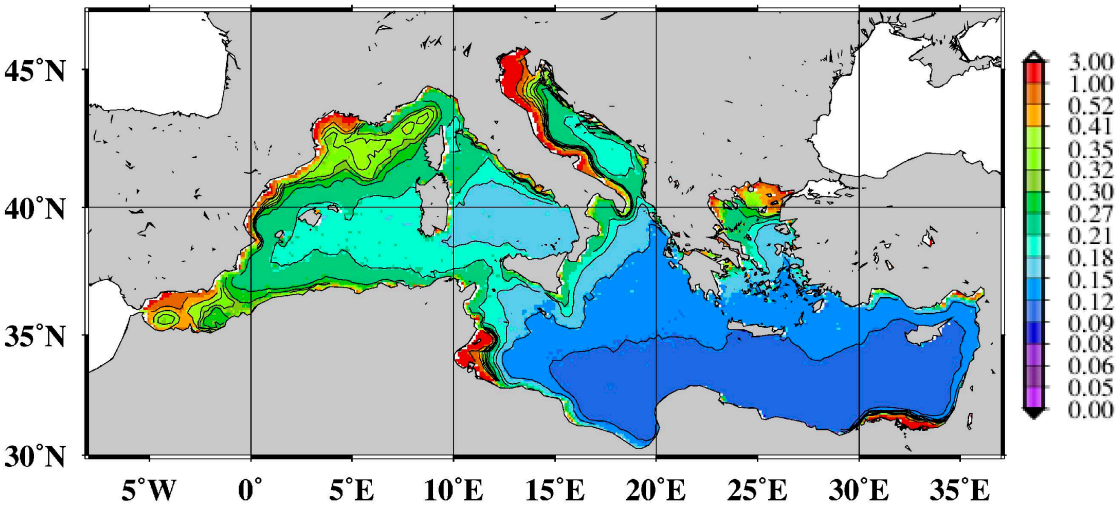

Figure 1.3. Distribution of mean annual satellite derived chlorophyll *a* concentration (mg m^{-3}) in the Mediterranean Sea. Figure taken from Siokou-Frangou et al. (2010) (modified from D'Ortenzio and Ribera d'Alcalá, 2009).

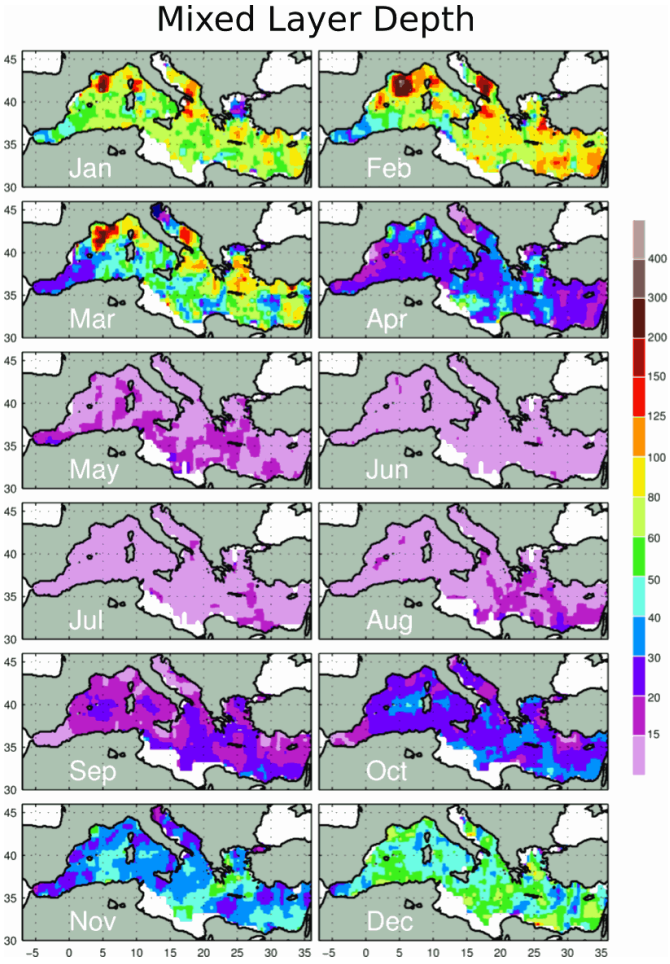

Figure 1.4. Depth of the mixed layer in the Mediterranean basin through the year. Figure taken from Houpert et al. (2015).

Concomitant to the west-east decreasing gradient of phytoplankton concentration and primary production, prokaryotic abundance and production (these two measurements include microorganisms from both the Archaea and Bacteria domains, but I will use the generic term "bacteria" instead hereafter, due to its more common use in the literature) also decrease from west to east (Siokou-Frangou et al., 2010). Reported values of bacterial abundance are within the range of 0.98 – 21×10^5 cell ml^{-1} in the western MS (Tanaka and Rassoulzadegan, 2002), 3 – 19×10^5 cell ml^{-1} in the noth Adriatic (Bongiorni et al., 2005), and around 3.8×10^5 cells ml^{-1} on average in the more oligotrophic eastern basin (Raveh et al., 2015; Wambeke et al., 2011). Bacterial

production in the western MS varies from minimum reported rates of 0.2 µg C l^{-1} d^{-1} (Pedrós-Alió et al., 1999) to 10 µg C l^{-1} d^{-1} (Gasol et al., 2012; Gomes et al., 2015). In the eastern basin instead, values as low as 0.0048 µg C l^{-1} d^{-1} were registered during a transect carried out in summer and a maximal rate of 1.96 µg C l^{-1} d^{-1} was recorded in September in the Aegean Sea (Siokou-Frangou et al., 2010, and references therein). Higher values of both bacterial abundance and production have also been found in coastal waters compared to offshore waters in both the western (e.g., Aguiló-Ferretjans et al., 2008) and the eastern (e.g., Raveh et al., 2015) basins, though. Siokou-Frangou et al. (2010) obtained similar slopes of linear regressions for bacterial biomass with respect bacterial production for the western and the eastern MS, in both cases being smaller than 0.4, thus pointing to a top-down control on bacteria (Billen et al., 1990; Ducklow et al., 1992). These authors also found a similar positive relationship between bacterial production and primary production in both regions, suggesting that primary production is an important source of dissolved organic carbon (DOC) for bacterioplankton. This relation has been found to be decoupled in the western MS (Turley et al., 2000) and in coastal waters of the eastern basin (Raveh et al., 2015), though, pointing to other sources of DOC when primary production is low.

As a consequence of the thermohaline circulation and the shallowness of the ML, nitrate (NO_3^-), phosphate (PO_4^{3-}) and silicate (Béthoux et al. 2002) are scarce in Mediterranean surface waters, especially during the stratification period, which extends from April – May to October – November (Estrada, 1985; Houpert et al. 2015). NO_3^- concentration range from < 0.5 µmol l^{-1} in the southern parts of the basin to ca. 5 µmol l^{-1} in the Gulf of Lion and the north of the Adriatic (Fig. 1.5a; Lazzari et al., 2016). PO_4^{3-} shows a clear west-to-east decreasing trend, with concentrations > 0.1 µmol l^{-1} in the northern part and close to 0 in the eastern basin (Fig. 1.5b; Lazzari et al., 2016; Segura-Noguera

et al., 2011). With an increasing nitrate to phosphate (N:P) ratio from ≤ 16 in the AW (Béthoux et al., 2002; Tanaka et al., 2011) to around 20 - 25 in the western region (Lazzari et al., 2016; Marty et al., 2002; Tanaka et al., 2011) and 25 - 30 in the eastern basin (Krom et al., 1991; Ribera d'Alcalà, 2003), Mediterranean waters are P-starved compared to other oceanic regions whose ratios of N to P are closer to the Redfield ratio of 16 (Redfield, 1934). The high N:P ratio in the MS has been suggested to be related either with a dust-derived stimulation of N_2 fixation by providing P and Fe, or because the high amounts of N compared to P introduced via external sources are not removed by denitrification either in the intermediate water or the sediments (Christodoulaki et al., 2016; Krom et al., 2004). The silicate (SiO_4^{4-}) to nitrogen ratio (Si:N) is also higher in the eastern (>1.3) than in the western basin (≤ 1) (Herrmann et al., 2013; Ribera d'Alcalà, 2003), SiO_4^{4-} concentration ranging from 0 to 5 μM in the western basin (Segura-Noguera et al., 2011) and from 1 to 7 μM in the eastern (Ribera d'Alcalà, 2003). Soluble iron (Fe) in Mediterranean surface waters is also high compared to P, ranging from 0.1 – 5 nM in the western basin, to 4 – 50 nM in the eastern (Guieu et al., 2010a), which is attributed to dust deposition. While N, P and Fe are essential for marine phytoplankton growth (and silicate in the case of diatoms), marine bacteria also need organic sources of carbon. The average DOC concentration in surface waters is around 80 μM in the MS, with an increasing trend towards the east (Avril, 2002; Pujo-Pay et al., 2011). In the coast, nutrient concentrations tend to be higher than in open waters, but they are highly variable at interannual and seasonal scales, depending on the contribution of external sources (i.e. river discharges and atmospheric deposition). Although P has often been considered the most important limiting nutrient in the Mediterranean, especially for bacterioplankton (Margalef et al. 1963, Marty et al. 2002; Siokou-Frangou et al., 2010; Lazarri et al. 2016), given the

overall low concentrations of nitrate and phosphate, the existence of N and P co-limitation has been reported in both the western and eastern basin at different times of the year, both in open and coastal waters (Guadayol et al., 2009; Moutin et al., 2012; Sala et al., 2006, 2002; Tanaka et al., 2011). On the other hand, the Mediterranean has been found to be limited in DOC during periods of winter mixing (December - March) either in open (Laghdass et al., 2012; Thingstad et al., 1997) or coastal (Pinhassi et al., 2006; Romera-Castillo et al., 2013) waters, thereby also restricting heterotrophic planktonic growth and production. Sala et al. (2002) reported that bacterioplankton of the oligotrophic MS lives in a dynamic equilibrium in which slight changes in grazing pressure, competition and nutrient concentrations can shift the communities from limitation by one nutrient to another.

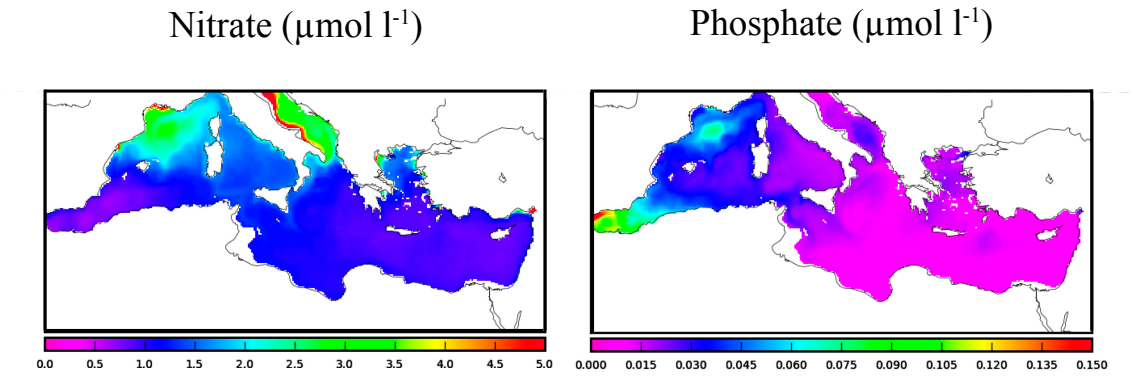

Figure 1.5. Spatial surface (0 – 50 m) distribution of nitrate (µmol l^{-1}) and phosphate (µmol l^{-1}) concentrations, averaged over the 1999-2004 period (modified from Lazzari et al. 2016).

The Mediterranean Sea is a unique marine system in terms of hydrography and biogeochemistry, in which most of the processes controlling the global ocean general circulation are present at reduced temporal and spatial scales. That is the reason why it has been considered as a "miniature ocean" or a "laboratory basin" (Lacombe et al., 1981; Robinson and Golnaraghi, 1995), being appropriate to study the interactions

between abiotic and biotic processes. Climatic models predict that the Mediterranean basin will be one of the regions most affected by the ongoing warming trend and by an increase in extreme events (Durrieu de Madron et al., 2011; Lionello et al., 2006), what makes the Mediterranean a potential model for global trends in marine biogeochemistry and biodiversity.

1.2 From the atmosphere to the ocean

The ocean and the atmosphere interchange gases, liquids, and particles suspended in gases (aerosols – I will use the terms "aerosols" and "atmospheric particles" as synonyms hereafter –). The global ocean has been considered a large sink of anthropogenic carbon dioxide (CO_2) (Takahashi et al., 2002) but, current model predictions of CO_2 exchange between the atmosphere and the ocean tend to find a loss in the ocean capacity as a sink of CO_2 as a direct consequence of ocean warming and thus a reduction in the power of the solubility pump (Bréviere et al., 2015). In the case of the Mediterranean, the eastern basin, due to its warm, stratified and nutrient-poor surface characteristics, act as a source of CO_2 (Sisma-Ventura et al., 2016; D'Ortenzio et al., 2008), while the western basin acts as a sink (D'Ortenzio et al., 2008). In all, D'Ortenzio et al. (2008), based on satellite-driven modeling of the upper-mixed layer, estimated that the MS, as a whole, acts as a small-to-medium sink for atmospheric CO_2 (0.24 Gt C y^{-1}). However, complex biological factors should also be considered to assess the net flux of CO_2 between the atmosphere and the ocean. Ridgwell and Arndt (2015) explain the CO_2 interchange between the ocean and the atmosphere by four conceptual pumps: the solubility pump, the carbonate pump, the organic carbon pump, and the microbial carbon pump. As in the present thesis we focus in the processes related with the biological part, I will only go into the detail for the last two processes,

illustrated in Fig. 1.6.

A. The *organic carbon pump* (Fig. 1.6a) considers the combined effect of the processes that involve (1) the fixation of CO_2 into organic matter (OM) through photosynthesis in the surface ocean, (2) consumption of that OM by heterotrophic organisms and its partial remineralization to CO_2, (3) physical mixing, and (4) transport and gravitational settling as particulate OM (Aparicio, 2016, and references therein).
B. The *microbial carbon pump* (MCP) (Fig. 1.6b). This concept was introduced by Jiao and coauthors (Jiao et al., 2010) and has been a focus of interest for the last years. The MCP emphasizes the role of bacteria, archaea and viruses in generating refractory DOM (RDOM) as a sub-product of the remineralization/respiration processes. Because of its recalcitrant nature, the RDOM can persist in the water column for centuries, thus acting as a reservoir of C. Alternatively, recent studies have shown that low DOC concentrations in the deep ocean, instead of its recalcitrant character, preclude its consumption by heterotrophic prokaryotes (Arrieta et al., 2015).

In oligotrophic regions, the labile components of dissolved OM produced in surface waters by phytoplankton are rapidly recycled in the surface layer and CO_2 is re-exchanged with the atmosphere (Karner and Herndl, 1992; Moutin and Raimbault, 2002), only a 10% of the OC (approximately 25.5 gC m^{-2} y^{-1}) being exported to the sea bottom in the NW Mediterranean (Herrmann et al. 2013). However, in Mediterranean surface waters, as in other oligotrophic regions of the world, large amounts of DOC (> 90 μM) have been observed during the stratification period either in coastal (Pinhassi et al., 2006; Romera-Castillo et al., 2013; Vila-Reixach et al., 2012) or open waters (Avril,

2002; Herrmann et al., 2013; Thingstad et al., 1997). Thingstad et al. (1997) suggested the existence of a "microbial loop malfunctioning" due to the lack of phosphate. Other studies instead point to the recalcitrant nature of the organic substrates that limits remineralization and favors the accumulation of "semi-labile" DOC (Carlson et al., 2002) (Fig. 1.6b). In the case that the first hypothesis is true, the effect of external sources of nutrients to the oligotrophic ocean should be considered, as they have the potential to relieve planktonic communities from nutrient limitation. The supply of nutrients will ultimately modify the structure of the food web and the relative abundance of species will influence how much CO_2 will be pumped to the deep ocean (Chisholm, 2000).

Despite the fact that oligotrophic regions, such as the Mediterranean, constitute more than 50% of the global ocean (Antoine et al., 1996; Longhurst, 1995), the biogeochemical impacts of atmospheric-derived nutrients on oligotrophic LNLC environments have received little attention in comparison to the large number of studies related to the impact of Fe supply on the productivity of high nutrient-low chlorophyll (HNLC) regions (Guieu et al. 2010b).

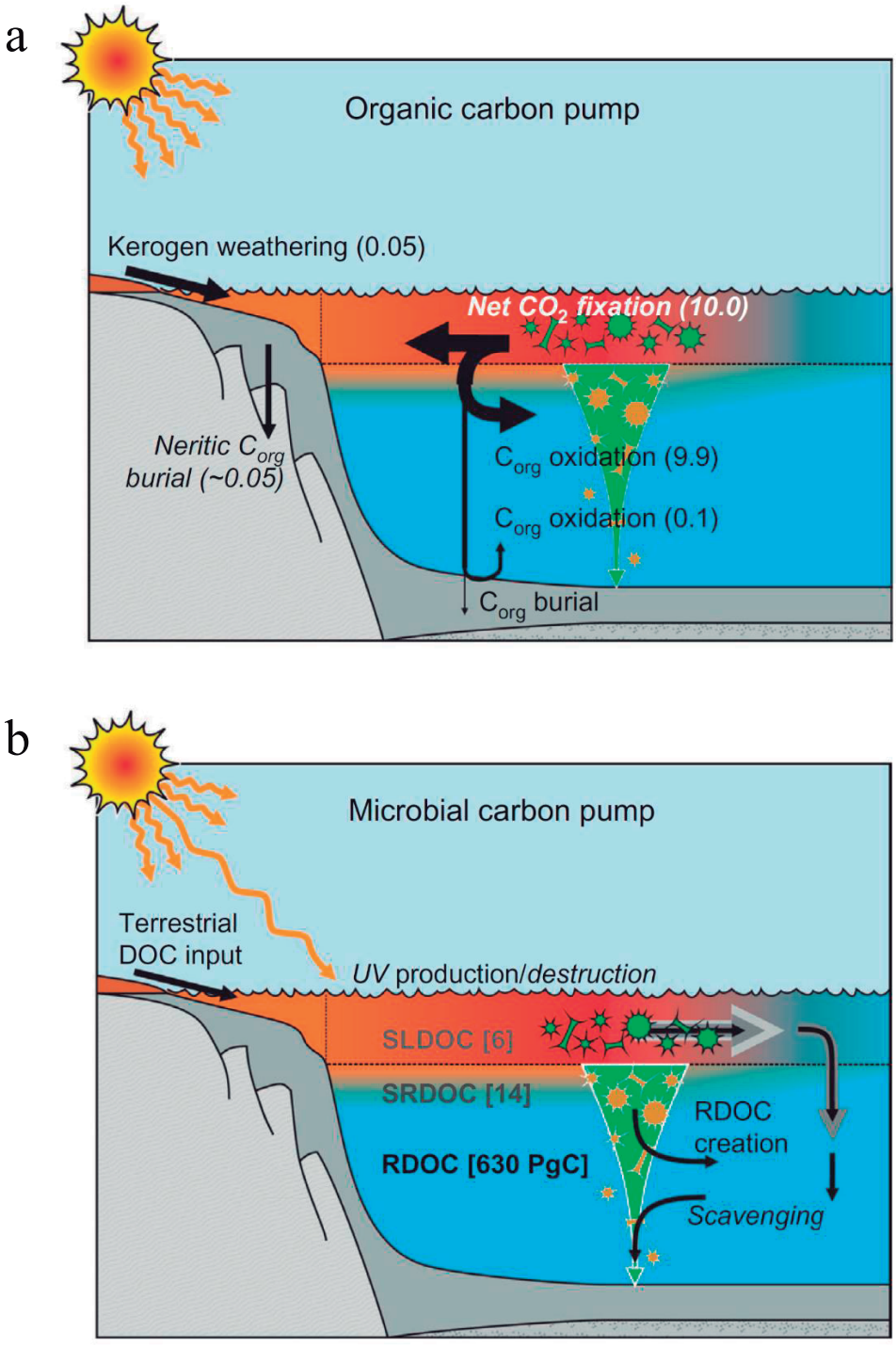

Figure 1.6. Scheme of the primary carbon reservoirs and processes constituting (a) the organic carbon pump and (b) the microbial carbon pump. Carbon fluxes (Pg C y^{-1}) into the ocean are shown in normal font and sinks in italics, and were quantified following Hönish et al. (2012). Reservoir inventories, in brackets, are from Hansell (2013). Figure taken from Ridgwell and Arndt (2015).

1.3 The atmosphere as a source of nutrients to Mediterranean surface waters: atmospheric deposition

The MS is surrounded by 24 countries and 46,000 km of coast. With about 420 million inhabitants in the countries around, 145 of them in the coastal area, it is continuously subjected to emissions of anthropogenic aerosols derived from agriculture, industry, and domestic activities (e.g., Durrieu de Madron et al., 2011; Lionello et al., 2006; Migon and Sandroni, 1999). On the other hand, the Saharan − the most important source of dust in the world (Dulac et al., 1996; Prospero and Lamb, 2003) − and Middle East deserts act as a source of crust-dominated aerosols (what is known as "dust") to the MS, which are transported in the form of non-continuous dust pulses, that can be observed from satellites (Fig. 1.7). Most annual dust deposition over the MS may occur in only a few days (Guerzoni et al., 1999). In opposition to the decreasing south-to-north trend of Saharan dust across the basin, anthropogenic atmospheric emissions decrease from the north to the south part of the basin (Barnaba and Gobbi, 2004; Pey et al., 2010; Volpe et al., 2009). However, while the implementation of European directives regarding particulate concentration and emissions to the atmosphere is improving air quality in the countries of southern Europe (Querol et al., 2014), the large population growth on the southern and eastern shores of the Mediterranean is currently producing an increasing anthropic pressure on the Mediterranean ecosystems, especially in the coastal locations (Lejeusne et al., 2010). In addition, the Mediterranean atmosphere receives atmospheric inputs from the Atlantic Ocean, from the sea surface, and from local volcanic activity (Guerzoni and al., 1997). Nevertheless, two principal sources, i.e. the European anthropogenic emissions and Saharan dust, dominate the atmospheric inputs to the basin. More precisely, Guerzoni et al. (1999), considered the aerosols transported to the MS to consist of anthropogenic-rich 'background' materials supplied continuously from

Europe, upon which sporadic pulses of Saharan crust-rich dust are superimposed.

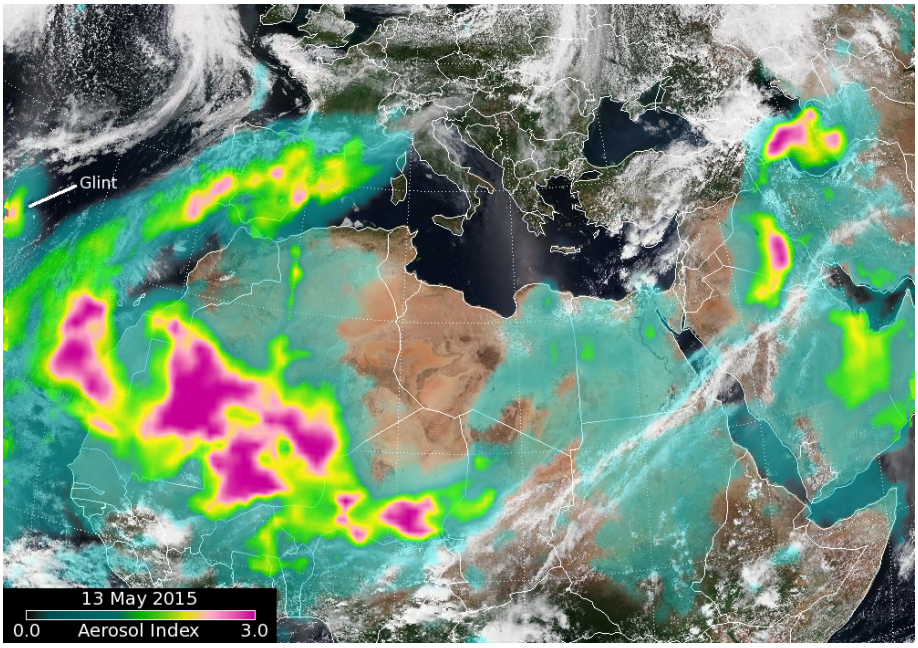

Figure 1.7. Saharan dust intrusion over the Iberian Peninsula on May, 13th, 2015. Taken from the Ozone Mapping and Profiler Suite (OMPS, NASA) website (https://ozoneaq.gsfc.nasa.gov/omps/blog/2015/05/).

Mean annual atmospheric aerosol load measured as PM_{10} (particles smaller than 10 μm) in Mediterranean locations range from values close to 15 μg m^{-3} in rural locations (e.g. Zarra, Spain, 436 inhabitants; Querol et al., 2009), to values up to 57 μg m^{-3} in urban locations (e.g. Barcelona, Spain, ca. 2 million inhabitants; Viana et al., 2005). Saharan dust outbreaks can increase these loads to > 100 μg m^{-3} (Querol et al., 2009). Guerzoni et al. (1999) reported mean annual deposition mass fluxes of total suspended particles (TSP) from the Sahara that ranged from 3–12 g m^{-2} y^{-1} in the western and 20 – 50 g m^{-2} y^{-1} in the eastern Mediterranean. In the eastern coast of Spain, Roda et al. (1993) and Carratala et al. (1996) estimated annual fluxes of African origin from 1 to 11 g m^{-2} y^{-1}. However, Saharan-dust events of high magnitude have been reported to leave daily depositions of more than 22 g m^{-2} in the western Mediterranean (Loÿe-Pilot and Martin, 1996; Ternon et al., 2010). High variability in particle deposition might be observed

during non-Saharan weather conditions as well, ranging from 0.8 to ca. 18 g m^{-2} y^{-1} in the NW Mediterranean (Pulido-Villena et al., 2008).

The chemical composition of Saharan dust over the Mediterranean atmosphere has been broadly characterized. The main components are the oxides of silicate (SiO_2; > 40%) and aluminum (Al_2O_3; about a third of silicate contribution), but calcium carbonate ($CaCO_3$), iron oxides (Fe_2O_3), nitrogen compounds, phosphorous, organic compounds and trace metals are also common and their contribution to the total particulate is highly dependent on the source area (Avila, 1997; Guieu and Thomas, 1997; Guieu et al. 2002; Moreno et al., 2006; Nava et al., 2012). In comparison with Saharan dust, anthropogenically-derived aerosols are characterized by a higher load of N and sulfate species, phosphates, carbonaceous species (organic and elemental carbon) and trace metals (Dall'Osto et al., 2010; Guerzoni et al., 1999; Migon et al., 2001; Pateraki et al., 2012; Querol et al., 2001; Rodríguez et al., 2002; Viana et al., 2005).

On the other hand, although anthropogenic particles are usually smaller (< 1 μm; Morawska et al., 2008; Moreno et al., 2015) than desert dust (2 μm on average; Arimoto et al., 1997; Jickells et al., 2005), both types of particles are small enough to be largely transported far away from their source, travelling for hundreds or even thousand of kilometers. During their transport, both types of aerosols are subjected to chemical interaction with other particles and with gases. As pollution involves high concentrations of NOx and SOx compounds, the main acid precursors species in the atmosphere (Stockdale et al. 2016), major elements and trace metals delivered from anthropogenic sources are much more soluble than those released by mineral dust, and thus more available for planktonic communities inhabiting surface waters of the ocean (Migon et al. 2001; Nenes et al. 2011; Jickells and Moore, 2015; Stockdale et al. 2016).

Finally, deposition of both types of particles in the land and the ocean may take place by wet (accompanied by rain) and dry deposition processes (Fig. 1.8).

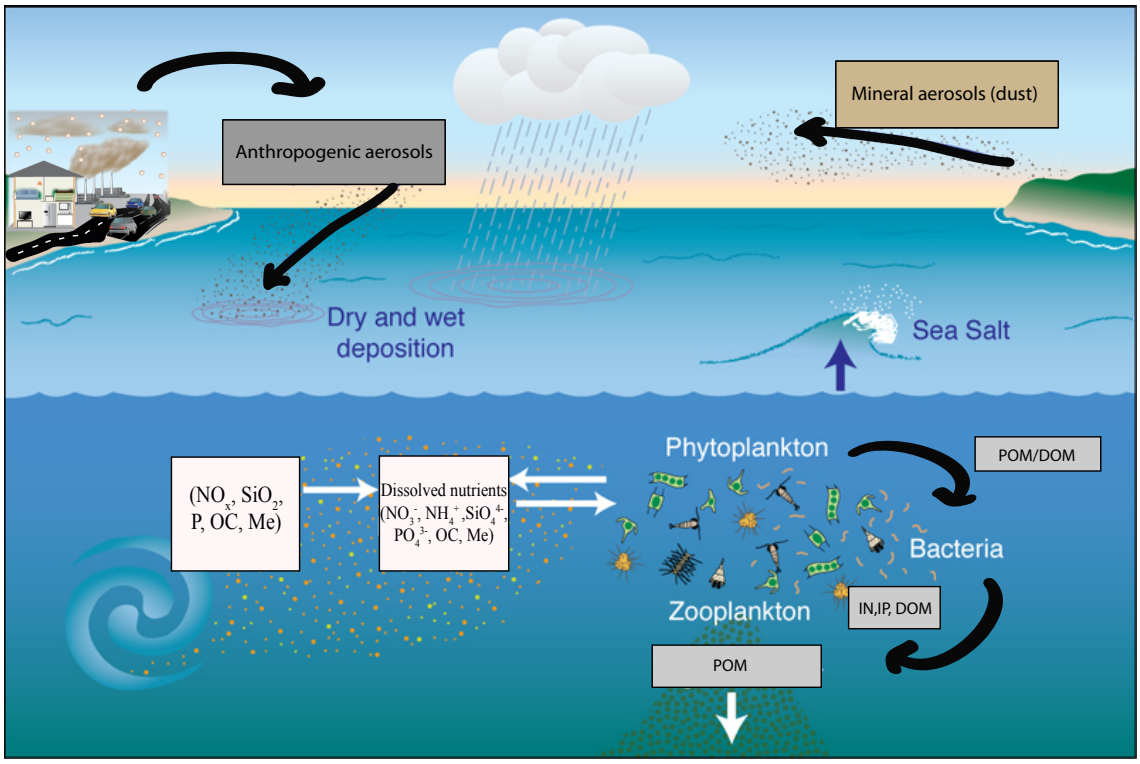

Figure 1.8. Scheme of dry and wet deposition of atmospheric particles from anthropogenic emissions and from the Saharan desert (dust). Part of the nutrients released from the atmosphere are dissolved in the seawater, being bioavailable for the planktonic community. Abbreviations: NOx = oxides of nitrogen, OC = organic carbon, Me = metals, IN = inorganic nitrogen (usually referred as the sum of nitrate, nitrite and ammonium), IP = inorganic phosphorous (mainly as phosphate), DOM = Dissolved organic matter, POM = Particulate organic matter. Modified from Sholkovitz et al. (http://www.whoi.edu/science/MCG/dept/facilities/sea_aer/maintextpg.html).

In spite of the importance of atmospheric deposition in biogeochemical cycling of nutrients in the Mediterranean, deposition data for N, P, OC and Si are scarce. Model data indicate that the atmosphere delivers more than half of total N and one-third of total P entering the whole basin (Guerzoni et al., 1999; Krom et al., 2004; Loÿe-Pilot et al., 1990). Measured data in sub basins, such as the NW Mediterranean and northern Adriatic, indicate an even greater proportion of atmospheric versus riverine inputs (Guerzoni et al. 1999). Loÿe-Pilot et al. (1992) and Copin-Montégut (1993) determined

that fluxes of OC from wet and dry deposition into the MS range from 10 to 20×10^{10} mol C y^{-1}. Atmospheric inputs of dissolved inorganic nitrogen (DIN; hereafter referred to as the sum of nitrate, nitrite, and ammonium) over the western basin are estimated to be 350×10^{3} t y^{-1}, very close to the total riverine input into the basin (403×10^{3} t y^{-1}) (Loÿe-Pilot et al., 1990). Krom et al. (2004), on the other hand, determined that atmospheric inputs of DIN and dissolved inorganic phosphorous (DIP; basically orthophosphate) accounted for 61% and 28% of the total budget of N and P, respectively, in the eastern Mediterranean. More precisely, Loÿe-Pilot et al. (1990) estimated DIN deposition fluxes of 29.2 mmol m^{-2} y^{-1} in the western basin, and that almost half of it was consequence of the human activity. Atmospheric deposition estimations from these authors are among the very few initially measured within the basin, and revealed the importance of atmospheric deposition in nutrient biogeochemical cycles and the lack of data in the MS. Then, more effort was put into measuring atmospheric deposition at different points of the basin through the ADIOS project (http://forecast.uoa.gr/adios/). Results from this project showed average DIN deposition fluxes of 33.2 mmol m^{-2} y^{-1} in the western MS, and 44.9 mmol m^{-2} y^{-1} in the eastern (Markaki et al., 2010). These authors estimated average DIP fluxes of 549 μmol m^{-2} y^{-1} in the western and 395 μmol m^{-2} y^{-1} in the eastern Mediterranean. While DIN deposition largely coincided with winter rainfall, DIP deposition was more variable throughout the year. Morales-Baquero (2013) also determined that most of the nitrate (two thirds) came from wet deposition in alpine lakes of the south-west of Spain, whereas Koçak (2015) found that half of the nitrate deposited from the atmosphere in a coastal location of Turkey came from dry deposition. Izquierdo et al. (2012) found that dry deposition accounted for ~50% of total particulate phosphorous (TPP) deposition at Montseny (NE of Spain; 40 km to the NNE of Barcelona), which amounts to 576

μmol m^{-2} y^{-1}, close to the flux determined by Markaki et al. (2010) in the western Mediterranean. African events were relevant in the annual budget (66% of TPP) but the dissolved fraction was higher in non-African events, according to their higher acidity. Measurements of the proportion of P released from dry with respect to wet deposition in the Mediterranean area oscillate between 23% (Koçak, 2015) to 80% (Pulido-Villena et al., 2008; Morales-Baquero et al., 2013). On the other hand, Markaki et al. (2010) also showed that the organic fraction in the atmospheric particles accounted for 38% of the total dissolved phosphorous (TDP) and 32% of the total dissolved nitrogen (TDN). However, most of these measurements were carried out in locations far from human activities. In coastal waters surrounding densely populated locations, a higher atmospheric deposition linked to anthropogenic emissions could be expected.

1.4 Future external sources of nutrients to the Mediterranean

Ludwig et al. (2009) found an overall decrease in the riverine freshwater discharge in the Mediterranean region, while PO_4^{3-} and NO_3^- discharges from rivers have significantly and moderately decreased, respectively, during the last two decades (Romero et al., 2013). In contrast, anthropogenic atmospheric deposition in the NW Mediterranean is expected to increase in the next decades as a consequence of the enhanced demographic pressure around the region, and the still increasing trends of anthropogenic pollutants in the northern hemisphere (Durrieu de Madron et al., 2011; Lionello et al., 2006). More specifically, Paerl (1995) estimated that the delivery of atmospheric N to coastal regions in Europe had increased by 50–200% during the past 50 years. Querol et al. (2014), on the other hand, found a downward concentration trend for PM_{10}, sulfate and NO_x in Spain during the last decade, attributed to the implementation of European directives on air quality and the economical crisis. Climate

models suggest that, in the future, European-sourced winds will be more prevalent over the MS than North African-sourced winds (McInnes et al., 2011). However, the presence of Saharan dust intrusions over the Mediterranean region is also believed to be more frequent and of higher magnitude in the following decades as a consequence of the increase in temperature (Fig. 1.9a) and a decrease in precipitation (Fig. 1.9b) in the region (Durrieu de Madron et al., 2011; Hoerling et al., 2012; IPCC, 2014; Prospero and Lamb, 2003). In fact, in the western Mediterranean, an increase of the frequency of rainfall loaded with dust (red rains) in the last decades has been evidenced (Avila and Peñuelas, 1999).

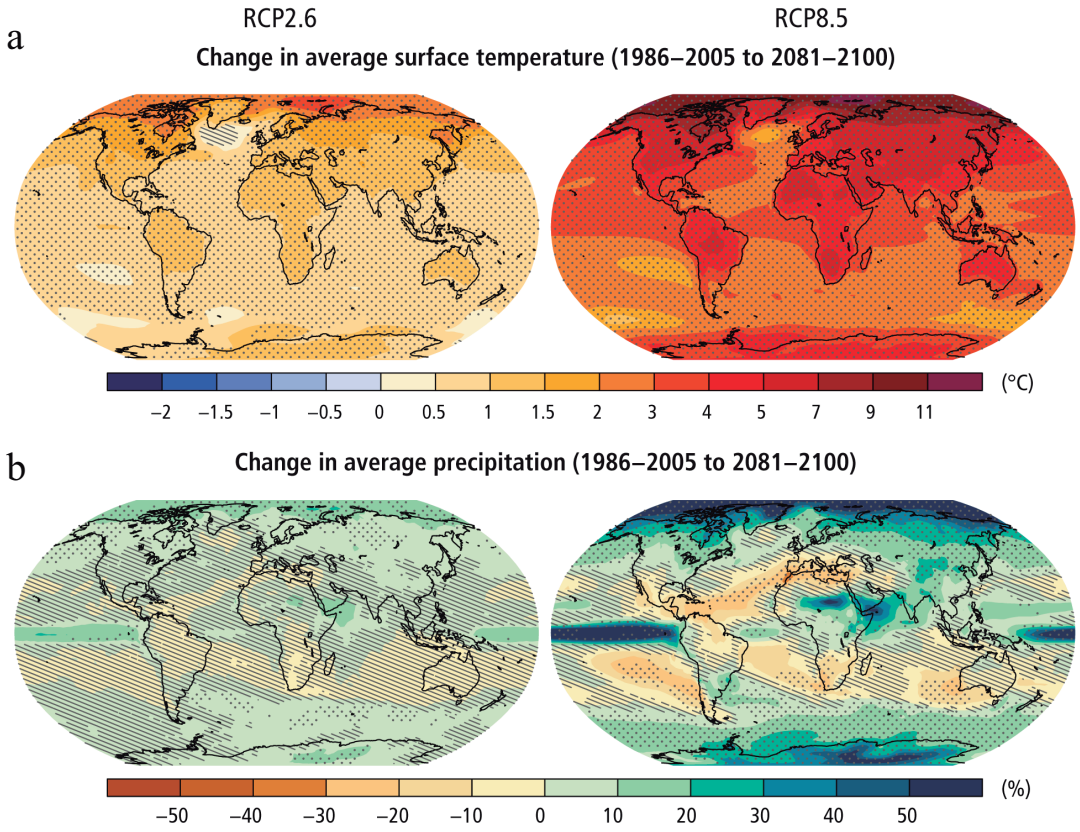

Figure 1.9. Changes in (a) average surface temperature and (b) average precipitation based on multi-model mean projections for the period 2081–2100 relative to the period 1986–2005. The left graphs show the expected changes under the stringent mitigation scenario that aims to reach emissions of CO_2 (Gt CO_2 y^{-1}) close to 0 by the year 2100 (RCP2.6), whereas right graphs contemplate the "worst" scenario, in which by 2100 anthropogenic emissions would exceed 1000 Gt CO_2 y^{-1} (RCP8.5). Figure taken from the IPCC report (2014).

In addition, the expected increase in the thermal stratification of the Mediterranean surface layer (Durrieu de Madron et al., 2011; Lionello et al., 2006) will reduce the supply of nutrients to the photic layer through vertical transport, enhancing the importance of external nutrient sources for the growth and production of planktonic microorganisms. In coastal waters of the MS, two types of climate-driven effects have already been observed: a warming trend and an increase in the frequency of extreme events (Lejeusne et al., 2010). Thus, it is becoming more important to assess the effect of atmospheric particles - both of mineral and anthropogenic origin - on the marine planktonic communities of the Mediterranean, especially so in coastal areas, more subjected to anthropic pressures. As phyto- and bacterioplankon are the base of the marine food web, changes in their dynamics and composition will ultimately affect the whole system.

1. 5 Impact of atmospheric deposition on the planktonic community

In spite of the overall oligotrophy in the MS, the supply of nutrients from external sources, such as atmospheric deposition and river discharges (Béthoux et al., 2002; Estrada, 1996; Ribera d'Alcalà et al., 2003), can relieve the planktonic community from nutrient limitation at certain times of the year. This yields to annual average primary production levels of 80 - 90 g C m^{-2} (Sournia et al., 1973), within the global oceanic annual mean fixation rates of 111 and 75 g C m^{-2} estimated by Bolin (1983) and Berger et al. (1987). While the relevance of both riverine runoff and atmospheric deposition to overall nutrient fluxes in the MS is still uncertain, budget calculations of atmospheric deposition suggest that atmospheric inputs support a significant amount of new production in Mediterranean surface waters (Guerzoni et al., 1999; Koçak, 2015; Markaki et al., 2003; Siokou-Frangou et al., 2010).

Because of its chemical composition (see Sect. 1.3) atmospheric deposition has been shown to play an important role in the cycling of nutrients in the MS (e.g., Guieu et al., 2014; Krom et al., 2004; Markaki et al., 2003; Nenes et al., 2011; Ridame and Guieu, 2002; Wuttig et al., 2013), and on the dynamics of the planktonic community. This is especially true during the stratification period, given the lower basal concentrations of N and P, and the higher frequency of Saharan events over the Mediterranean. More specifically, Saharan dust intrusions are more frequent in spring over the eastern basin and in summer in the western (Barnaba and Gobbi, 2004; Querol et al., 2009; Volpe et al., 2009). Atmospheric anthropogenic inputs, although of lower magnitude, can also be relevant when surface waters are nutrient depleted, supplying soluble N and P. Anthropogenic aerosols have also the potential to stimulate bacterial growth during winter, as pollution episodes from Europe are more frequent at this time of the year (Pey et al., 2010; Viana et al., 2005) and surface waters may be limited by OC, as previously mentioned.

In coastal waters, albeit annual atmospheric bulk deposition may be relatively low in comparison to average nutrient concentrations in surface waters, often nutrients may be temporarily unbalanced and even at extremely low concentrations. For instance, Arin et al. (2013) determined minimum concentrations of total inorganic nitrogen (TIN) as low as 0.04 μM during summer and 0.01 μM of phosphate in autumn in Barcelona coastal waters. Koçak (2015) reported that, during the stratification period, atmospheric P input might sustain 80 % of the new production in a coastal location of Turkey, whereas, atmospheric N input might support 8 times higher new production than that detected for surface waters. Moreover, in coastal waters (especially of urban locations), contrary to what happens in open waters, where chlorophyll values and plankton dynamics can be predicted with a reasonable accuracy on an annual basis, biological parameters often

show slight seasonality, as they are exposed to numerous and convergent forcing factors that make it difficult to draw clear patterns (Romero et al., 2014).

The effect of Saharan dust on marine nutrient chemical cycles and it is impact on the planktonic community inhabiting the surface waters of the open ocean and coastal waters has recently been focus of attention. The biogeochemical effect of aerosols in oligotrophic waters has been explored through micro- and mesocosm experiments where different combinations of nutrients or Saharan dust (and in only few cases anthropogenic aerosols) have been added to natural planktonic assemblages. As under nutrient stress conditions phytoplankton and heterotrophic bacteria compete for nutrients (mainly N and P in the Mediterranean), the response is expected to differ as a function of the biogeochemical status of the seawater. In some of these amendment studies, phytoplankton growth (usually measured as chlorophyll *a*) is a first observed response to the aerosol addition (Bonnet et al., 2005; Duarte et al., 2006; Herut et al., 2005; Laghdass et al., 2011). Subsequently, heterotrophic bacteria may be indirectly stimulated, remineralizing the organic matter produced by the phytoplankton. In other experiments instead, bacterial growth and production showed a quicker stimulation (Lekunberri et al., 2010; Pulido-Villena et al., 2008; Romero et al., 2011). In this case, primary production may also be secondarily stimulated by the recycling of inorganic nutrients. This indirect effect on primary producers may be weaker than a direct stimulation and may imply a delay in time, which may range weeks in the ocean, all of which complicates the detection of aerosol effects on plankton dynamics at the system level. As stated by Marañón et al. (2010), the overall response could depend on the oligotrohy of the region. Raven (1998) hypothesized that bacteria are better competitors in oligotrophic waters, given their larger surface:volume ratio. In contrast, Goldman (1993) showed that diatoms tend to be competitively superior in nutrient-replete

environments because they are more capable of responding quickly to nutrient injections. This may explain the good correlation observed between increasing aerosol concentration and chlorophyll response in experiments (Table 1.1 and Fig. 1.10). In contrast, bacterial abundance shows no clear pattern with respect aerosol concentration, and a slight trend to decrease with incubation time (Fig. 1.10). Bacteria sometimes show a quick response but decrease towards the end of the experiment, coinciding with an increase in heterotrophic nanoflagellates (Romero et al., 2011; Pitta et al., 2017) or viruses (Pulido-Villena et al., 2014). However, a higher response has usually been observed in bacterial production and respiration rates in comparison to bacterial abundance (Duarte et al., 2006; Herut et al., 2016, 2005; Lekunberri et al., 2010; Marañón et al., 2010; Pulido-Villena et al., 2014).

Table 1.1. Summary of the responses observed in chlorophyll (CHL) and bacteria (BAC) concentration in previous aerosol-addition experiments carried out in the Mediterranean Sea. The response is expressed as the ratio between the maximal increase in the aerosol treatment with respect to the controls. A dash indicates no observed stimulation (ratio ≤ 1). NA = not assessed. The asterisks denote experiments where anthropogenic aerosols were used.

AEROSOL (mg l^{-1})	CHL	BAC	TIME (h)	Reference
0.01	1.6	1.1	80	Bonnet et al. (2005)*
0.01-0.03	1.9	1.1	48	Ternon et al. (2011)*
0.2	1.1	1.2	90	Herut et al. (2005)
0.25	1.4	1.1	80	Bonnet et al. (2005)
0.5	NA	1.3	72	Pulido-Villena et al. (2008)
0.7	1.4	1.3	90	Herut et al. (2005)
0.8	1.5	1.1	72	Laghdass et al. (2011)
0.8	1.8	-	192	Laghdass et al. (2011)
0.8	2	1.3	24	Pulido-Villena et al. (2014)
0.8	2.4	-	144	Pulido-Villena et al. (2014)
1	1.2	-	48	Ternon et al. (2011)
1	1.7	1.5	192	Herut et al. (2016)*
1.3	1.6	1.4	48	Pitta et al. (2017)
1.6	1.6	1.4	192	Herut et al. (2016)
2	NA	2.3	72	Pulido-Villena et al. (2008)
2	2.5	1.7	90	Herut et al. (2005)
5	4.8	1.2	90	Herut et al. (2005)
50	1.5	2.1	72	Romero et al. (2011)
500	5	12.5	72	Romero et al. (2011)
50	2.7	1.5	168	Romero et al. (2011)
500	11	2	168	Romero et al. (2011)

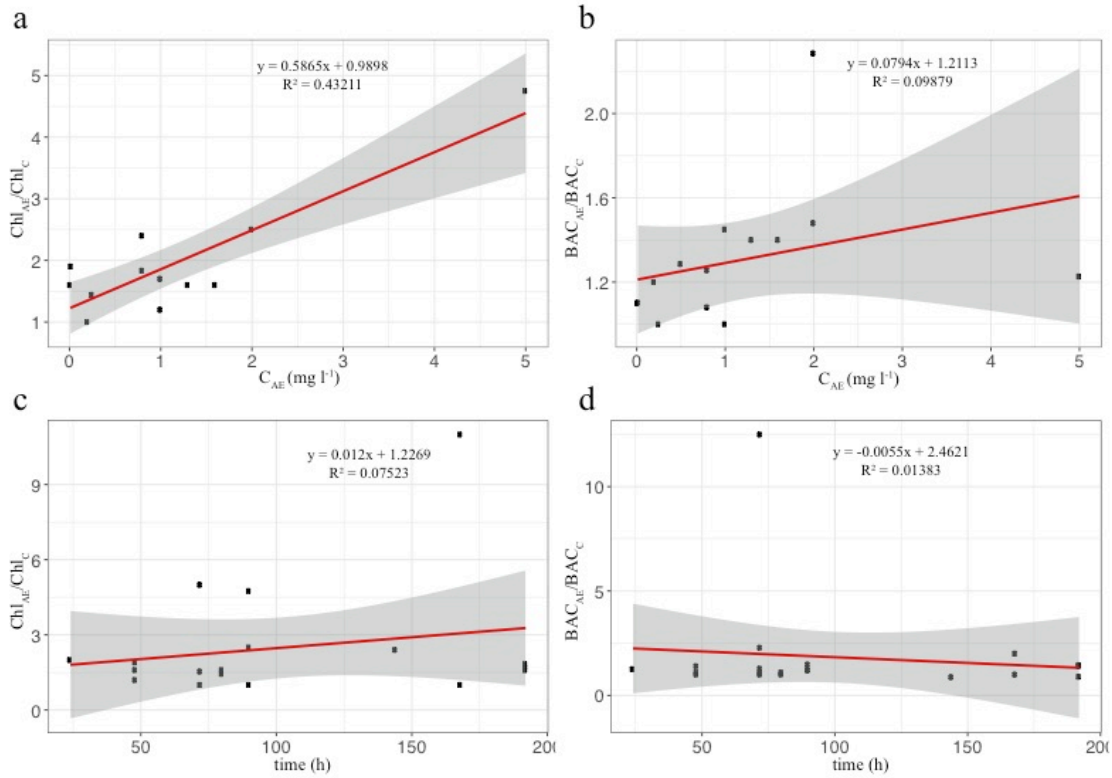

Figure 1.10. Response of chlorophyll and bacteria − as the ratio of the concentration in the aerosol treatments respect to that in the controls − with respect to the aerosol concentration (a, b) and the incubation time (c, d). The grey shading delimits the confidence interval (95%). References in Table 1.1 were used for the correlations (data from Romero et al. 2011 were removed for the relationship with the aerosol concentration).

Moreover, some of these studies have shown that different phytoplankton and heterotrophic bacterial groups do not respond equally to aerosols, often showing changes during the incubation time (Laghdass et al., 2011; Lekunberri et al., 2010; Marañón et al., 2010; Paytan et al., 2009; Romero et al., 2011). Whereas phytoplankton composition may be determined by microscopy, changes in the composition of bacterial assemblages are more difficult to assess. Molecular tools are currently providing more information about changes in the heterotrophic bacterial communities with respect to nutrient conditions in the Mediterranean Sea (e.g., Laghdass et al., 2011; Lami et al., 2009; Pinhassi et al., 2006, 2004), and therefore are a valuable tool to study the effect of aerosols on bacterial assemblages. In the past, environmental genetic analyses were

restricted to specific well-known phylogenetic groups by means of fluorescence in situ hybridization (FISH) or denaturing gradient gel electrophoresis (DGGE). Studies assessing bacterial community composition when subjected to Saharan dust were based on these techniques (Laghdass et al., 2012; Lekunberri et al., 2010; Marañón et al., 2010; Pulido-Villena et al., 2014; Reche et al., 2009). Although results do not show a clear pattern and these techniques do not allow a high taxonomic resolution, some of these studies show that dust might stimulate certain bacterial groups over others by providing specific nutrients. Nowadays, next-generation sequencing methodologies allow obtaining results at a much higher taxonomic resolution. Only one recent study is known in which the effect of both anthropogenic and Saharan aerosols have been evaluated by means of 454-pyrosequencing (Guo et al., 2016). Overall, these studies tend to show that different members of the groups *Alphaproteobacteria*, *Gammaproteobacteria,* and *Bacteroidetes*, are among the main favored with aerosol additions, but the variability observed within groups is high.

On the other hand, the effect of anthropogenic aerosols on marine bacteria remains poorly studied. Only Bonnet et al. (2005) and Ternon et al. (2011) studied the effect of anthropogenic aerosols on bacterioplankton abundance in Mediterranean surface waters, and found almost no response (Table 1.1). Some studies in the eutrophic coastal waters of the NW Iberian Peninsula have explored the effect of rainwater from different sources on the heterotrophic community, though. These studies have shown a low effect on bacterial abundance as well, but a large effect on bacterial production and respiration (Martínez-García et al., 2015; Teira et al., 2013). They also studied the effect of rainwater on the bacterial community composition and found an overall positive response of *Betaproteobacteria* and *Bacteroidetes* to rain additions, and also of some groups of *Alpha-* and *Gammaproteobacteria* at certain times of the year. Recently,

Herut et al. (2016) and Guo et al. (2016) have evaluated the effect of both anthropogenic and Saharan aerosols on bacterial metabolism and composition in the eastern Mediterranean, showing major changes with anthropogenic aerosols. However, to our knowledge, the effect of anthropogenic aerosols on bacterial activity and composition has not been evaluated yet in coastal waters of the Mediterranean.

On the other hand, aerosol particles also contain elements such as metals (Fe, Cu, Cd, Zn, Mn, etc.) that are essential in trace amounts for plankton physiology but can be toxic at high concentrations. The effects depend on the characteristics of the metals, the cells that receive them and other physical factors, such as light (Mann et al., 2002; Sunda and Huntsman, 2004, 1998). Some experiments carried out in the oligotrophic Red Sea (Paytan et al., 2009) and Sargasso Sea (Khammeri et al., unpubl. data) have shown a negative impact of metals contained in aerosol samples to some species of phytoplankton. Jordi et al. (2012) also showed negative relationships between high amounts of atmospheric Cu and chlorophyll dynamics in the western MS, but this has not been experimentally tested up to date.

1.6 The North-Western (NW) basin: our area of study

The work presented in this doctoral thesis is focused in the NW Mediterranean sub-basin (see Fig. 1.1). Throughout the thesis, we aim to elucidate the effect of atmospheric particles with a markedly different origin (i.e. Saharan dust *versus* anthropogenic aerosols) on marine planktonic communities of two coastal sites with a contrasted anthropogenic footprint. The work was mainly carried out in two locations of the Catalan coast, namely Barcelona (41° 23´ N, 2º 11´ E) and Blanes (41° 40´ N, 2º 48´ E) (Figure 1.11). These locations have been selected because of their historic backbone time series and also because, despite they are only separated by 70 km, Barcelona is

more anthropogenically influenced with somewhat higher nutrient and organic carbon load (both from terrestrial and atmospheric sources) (Table 1.2), whereas the coastal plankton in Blanes is relatively unaffected by human influence (Gasol et al., 2012). Viana et al. (2005) showed daily PM_{10} levels in Barcelona that ranged from 31 μg m^{-3} at the Fabra observatory (400 m. ASL) to 57 μg m^{-3} at the Harbor (sea level), consistent with annual mean values of 39 μg PM_{10} m^{-3} measured by Pey et al. (2008) and 49.5 μg m^{-3} by Rodríguez et al. (2002). In contrast, mean PM_{10} levels of 16 – 18 μg m^{-3} have been determined at the Montseny rural site (41° 46´ N; 02° 21´ E, 720 m ASL) by Pey et al. (2009) and Querol et al. (2009), which could be representative of atmospheric conditions in Blanes, although Blanes is more affected by sea-spray and traffic and boat emissions than the Montseny site. Although both coastal locations are fairly well characterized with respect to nutrient and phytoplankton concentrations (Arin et al., 2013; Gasol et al., 2012; Guadayol et al., 2009; Pedrós-Alió et al., 1999; Romero et al., 2014), and bacterial concentration, activity and composition (Alonso-Sáez et al., 2007; Gasol et al., 2012; Pedrós-Alió et al., 1999; Sala et al., 2006, 2002; Schauer et al., 2003) (Table 1.2), only one study has attempted to assess the effect of Saharan dust in the coastal waters of Barcelona (Lekunberri et al., 2010; Romero et al., 2011), whereas no studies in the area have investigated the effect of anthropogenic particles on marine planktonic communities.

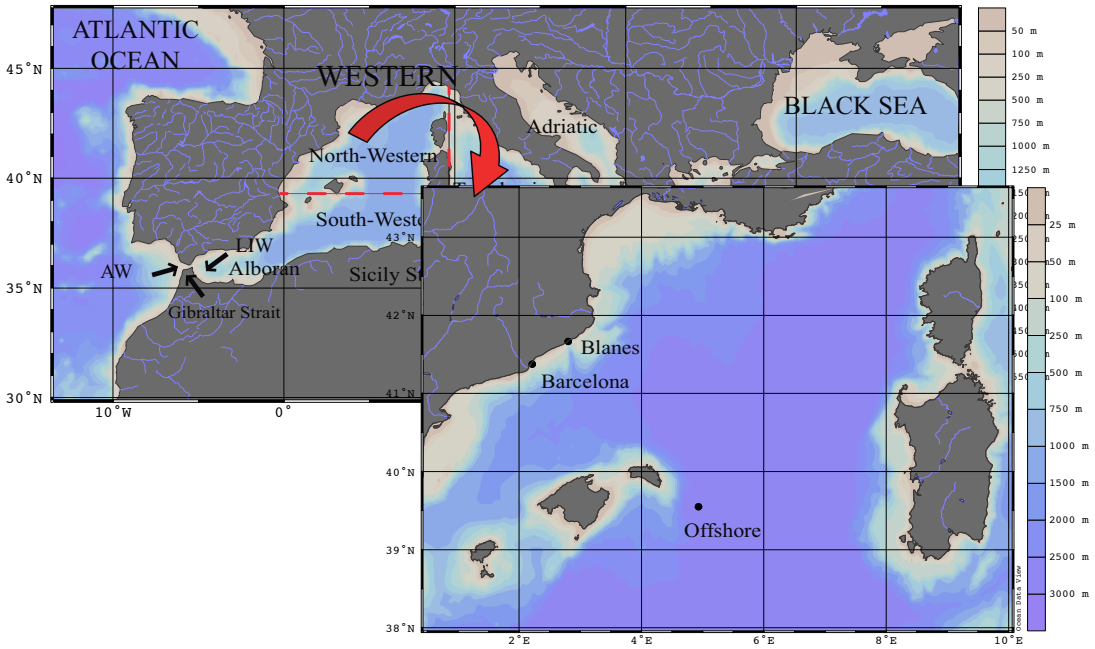

Figure 1.11. Map of the area of study within the NW Mediterranean sub-basin. Bathymetry is signaled on the right colored legend.

In addition to these two coastal sites, we have selected a location offshore the Balearic Islands (39° 33′ N; 04° 56′ E) (Fig. 1.11), with the aim to compare the effect of the two types of aerosols in a more pristine environment with a higher degree of oligotrophy. Querol et al. (2009) estimated a PM_{10} concentration of 24 μg m^{-3} over Bellver (Balearic Islands, Spain), but a lower value could be expected over our remote station. In comparison with Barcelona and Blanes coastal waters, biogeochemical data for this station is much more scarce. Satellite images and models, though, allow to describe the main characteristic of the location to a certain extend (Table 1.2). In contrast to NW Mediterranean coastal waters, that reach the highest levels of phytoplankton biomass and productivity in the basin (Fig. 1.3 and Table 1.2), offshore waters are much more depleted in chlorophyll, maximal concentrations reaching no more than 0.5 mg m^{-3} (D'Ortenzio and Ribera D'Alcalà, 2009; Gallisai et al., 2014; Volpe et al., 2009, 2007; Fig. 1.3). Accordingly, higher bacterial density and production have been reported in

coastal waters than in offshore waters of the NW Mediterranean. For instance, Pedrós-Alió et al. (1999) found bacterial abundances of around 7×10^5 cell ml^{-1} close to the coast of Barcelona and around 4×10^5 cell ml^{-1} close to Balearic Islands during summer, and integrated bacterial production in the euphotic zone was almost 5 times higher in the coastal station close to Barcelona.

Table 1.2. Main features of the three stations of study within the NW Mediterranean region. When known, the annual average is given, followed by the minimum and the maximum data reported in the literature (in parenthesis).

	BARCELONA[a]	**BLANES**[f]	**OFFSHORE**
Latitude (º N)	41° 22´	41º 40´	39º 33´
Longitude (º E)	2º 12´	2º 48´	4º 56´
Depth (m)	40	20	2655
PM_{10}	31 – 57[b]	16 – 18[g]	21 – 24[g]
NO_3^- (µM)	1.04(<0.015 – 21.91)	1.45(0.01 – 4.43)[g]	2.11[k](0.34[e] – 2.74[k])
NH_4^+ (µM)	1.92 (<0.02 – 57.92)	0.22[h] (0.04[h] –1)	0.16[e]
PO_4^{3-} (µM)	0.19 (0.01 – 2.09)	0.12[h] (0.02 – 0.5)	0.04 (<0.02 – 0.07)[k]
$Si(OH)_4$ (µM)	0.9 (<0.017 – 12.47)	2.35 (0.07 – 5.93) [h]	0.57[e]
DOC (µM)	88 (61 – 149)[c]	79 (50 – 135)[i]	81[e]
Chl (µg l^{-1})	1.06 (0.1 – 6.63)	0.63 (0.1 – 1.8)[i,j]	0.15 (0.06 – 0.31)[e,l]
HBA (cell ml^{-1}) ×10^5	12.5 (6.4 – 21.2)	7 – 12	3.7 – 5[d,e]
HBP (µg C l^{-1} d^{-1})	1[d] – 4.53[e]	1 – 10	0.4[e] – 0.5[m]

[a] From Arin et al. (2013) and Romero et al. (2014) unless other is specified; [b] Viana et al. (2005); [c] unpublished data; [d] Pedrós-Alió et al. (1999). These data were collected only during the summer period in a transect from Barcelona to the Balearic Islands, therefore values for HBA offshore are not really from our station but from the Catalano-Balearic Sea. [e] This study. These data come from punctual observations and may not be representative of a given location. [f] From Gasol et al. (2012) unless other is specified; [g] Querol et al. (2009); [h] Guadayol et al. (2009); [i] Romera-Castillo et al. (2013); [j] Vila-Reixach et al. (2012); [k] Lazzari et al. (2016); [l] Lazzari et al. (2012); [m]Wambeke et al. (2011).

1.7 Aims of the thesis

This thesis dissertation is conceived to provide a better understanding of the role of atmospheric deposition as a source of nutrients in the oligotrophic ocean. We collected atmospheric deposition at two sites that differ in their anthropogenic footprint and estimated the potential fertilizing effect in the water column. At the same time, we directly measured the impact of anthropogenic atmospheric inputs with respect to Saharan dust using amendment microcosm experiments and focusing mainly on marine bacterioplankton. These approaches were followed to answer three main questions:

1) What are the spatiotemporal chemical characteristics of anthropogenic and natural aerosols depositions in the Mediterranean coastal region? (assessed in Chap. 2);

2) How do atmospheric particles (from mineral and anthropogenic sources) in these coastal environments influence both the autotrophic and heterotrophic planktonic communities at different times of the year, with different initial biogeochemical conditions in the seawater? (assessed in Chap. 3);

3) How do atmospheric particles (from mineral and anthropogenic sources) influence marine bacterioplankton abundance, composition, and activity, depending on the initial status of the seawater? Have aerosols the potential to affect bacteria directly or there is a needed mediation through phytoplankton growth? (assessed in Chap. 4 and 5).

Chapters 3, 4 and 5 present results from six microcosm experiments carried out in three different locations of the NW Mediterranean and at different times of the year. Three of them were carried out in Barcelona (during winter, spring, and summer), two of them in Blanes (spring and summer), and another in the offshore station during summer (se Fig. 1.12 for an overview of the experimental design). The methodology used for aerosol collection, characterization, and inoculation into the experimental containers is fairly

described through these three chapters, so I will not go into detail now. Briefly, we added aerosols of Saharan ("S") or anthropogenic ("A") origin into duplicate microcosms that contained seawater from one of the three locations. The concentration used for the amendments was 0.8 mg l^{-1}, corresponding approximately to a medium-high deposition event of 8 g m^{-2} into a column water of 10 m, the typical depth of the mixed layer during most of the year in the western Mediterranean (D'Ortenzio et al., 2005; Houpert et al., 2015) (Fig. 1.4). Other two (or four) microcosms were left without atmospheric particles, serving as controls (Fig. 1.12). We then followed the dynamics of inorganic and organic compounds, phytoplankton, bacterioplankton and heterotrophic nanoflagelates in the experimental containers for several days (from 2 to 7 depending on the experiment).

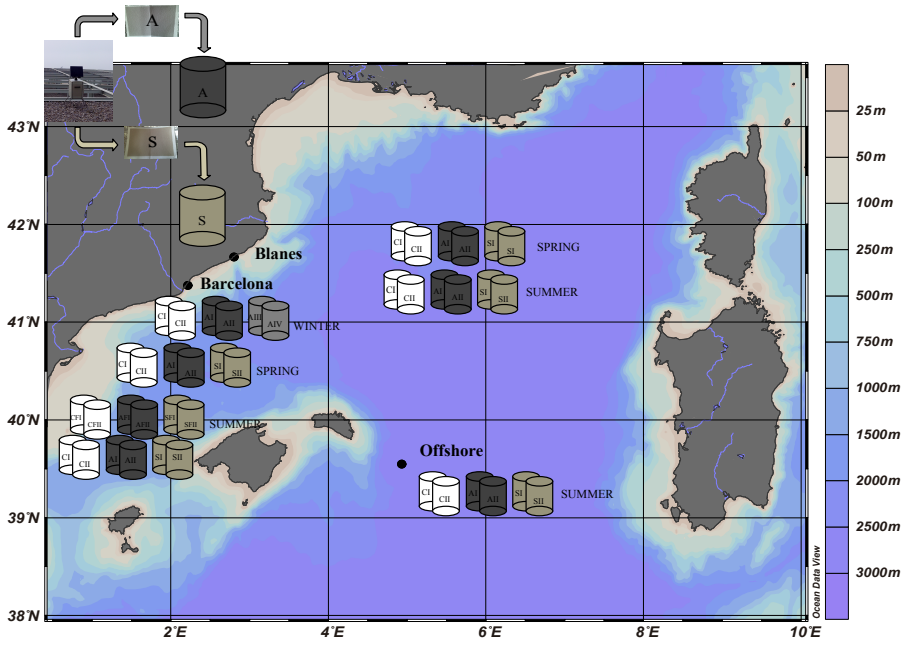

Figure 1.12. Scheme of the main set-up used for the microcosm experiments. Atmospheric particles of Saharan (S) and anthropogenic (A) origin were collected on quartz fiber filters (top left corner). The basic design consisted of two containers amended with Saharan dust (SI and SII), two with anthropogenic particles (AI and AII), and other two controls (CI and CII). In the winter experiment, there were no Saharan samples but two containers amended with one type of anthropogenic particles (AI and AII), and other two with anthropogenic particles of different composition (AIII and AIV). In the summer experiment of Barcelona, we used other 6 containers with seawater further filtered by 0.8 µm (CFI, CFII, AFI, AFII, SFI, SFII).

Specific objectives of the thesis are addressed in different chapters:

Chapter 2. Atmospheric deposition in two coastal locations with contrasted anthropogenic footprint.

We have measured the atmospheric deposition fluxes of inorganic nutrients (nitrate, nitrite, ammonium, phosphate and silicate) and organic carbon in two coastal locations of the NW Mediterranean with contrasted anthropogenic footprint (i.e. Barcelona and Blanes). The time-series was carried out during 4.5 years in Barcelona and for 3 years in Blanes. We explored the relation between nutrient deposition and meteorological variables measured in both stations. We compare our results with those from other locations in the Mediterranean and discuss the implications for the planktonic communities.

Chapter 3. Anthropogenic versus mineral aerosols in the stimulation of microbial planktonic communities in coastal waters of the northwestern Mediterranean Sea.

In this chapter we compare the effect of aerosols of anthropogenic origin and Saharan dust on different compartments of the planktonic community (phytoplankton of different sizes, bacteria and heterotrophic nanoflagellates) inhabiting the surface waters of an urban location (i.e. Barcelona). We test the effect of aerosols at different times of the year, and discuss about the final balance in autotrophic *versus* heterotrophic enhancement with atmospheric particles.

Chapter 4. The origin of atmospheric particles determines their effect on coastal bacterioplankton metabolism.

Here we assess the effect of both anthropogenic and mineral particles on prokaryotic abundance and activity, at different times of the year, and in the three locations during the summer stratification period. Atmospheric particles were collected in the same location where they were subsequently added into the microcosms (Barcelona or Blanes), with the aim to test the effect of local atmospheric sources on coastal bacterioplankton. We discuss the relationship between the nutrients released by the aerosols and the observed response on the prokaryotic community.

Chapter 5. Atmospheric deposition shapes bacterial community assembly in the northwestern Mediterranean.

In this chapter we focus on the effect of aerosols on bacterial community composition, considering both the autotrophic (*Cyanobacteria*) and heterotrophic members. We compare the effect of both types of aerosols in the three locations and at different times of the year.

Chapter 6. General discussion and perspectives.

Here we will discuss the results obtained in comparison to the available literature, and suggest further steps in the research of the assessment of effects of atmospheric particles on the microbial planktonic community.

Chapter 2

Atmospheric deposition in two coastal locations with contrasted anthropogenic footprint

Isabel Marín, Rachele Gallisai, Francisco L. Aparicio, Riccardo Leardi, Cèlia Marrasé, Xavier Querol, Pep Gasol, Francesc Peters (in prep.)

2.1 Introduction

The effect of atmospheric deposition from mineral and anthropogenic sources on the biogeochemistry of the Mediterranean Sea has become a topic of interest during the last two decades, as it has been shown that the contribution of atmospheric inputs in terms of biologically-available nitrogen (N), phosphate (PO_4^{3-}), and silicate (SiO_4^{4-}) to this oligotrophic basin can account for more than 50% of the total inputs at certain times of the year (Guerzoni et al., 1999; Krom et al., 2004; Markaki et al., 2010; Pateraki et al., 2012). In coastal areas of the Mediterranean, atmospheric deposition of inorganic nutrients and trace metals may even surpass river discharges (Durrieu de Madron et al., 2011; Koçak et al., 2010; Krom et al., 2004; Martin et al., 1989). As these nutrients are essential for marine microorganisms, an ultimate effect on the growth and activity of both the autotrophic (Bonnet et al., 2005; Herut et al., 2016; Laghdass et al., 2011; MacKey et al., 2012; Romero et al., 2011; Ternon et al., 2011) and heterotrophic (Herut et al., 2005, 2016; Pulido-Villena et al., 2008, 2014; Reche et al., 2009) planktonic communities has been observed.

In the western Mediterranean Sea (WMS), PO_4^{3-} and N are the main limiting nutrients for marine production (e.g., Migon and Sandroni, 1999; Moutin et al., 2012; Sala et al., 2002). Surface waters are also poor in silicate, though, pointing that diatom production might also be limited by the availability of this nutrient (Béthoux et al., 2002). In addition, it has been reported that organic carbon (OC) can co-limit bacterial production at certain times of the year in the Mediterranean basin (Romera-Castillo et al., 2013; Thingstad et al., 1997). Still, data of atmospheric deposition fluxes of essential nutrients as N, PO_4^{3-}, SiO_4^{4-} and OC are scarce. In the WMS, most of the studies have focused in the deposition of N or PO_4^{3-}, usually measured in remote areas of the basin (Avila and

Rodà, 2002; Izquierdo et al., 2012; Markaki et al., 2010; Migon and Sandroni, 1999; Pulido-Villena et al., 2008), or high mountain lakes of the Iberian Peninsula (de Vicente et al., 2012; Morales-Baquero et al., 2006; Reche et al., 2009). De Vicente et al. (2012) and Pulido-Villena et al. (2008) also measured the atmospheric deposition of OC, whereas the study of Morales-Baquero et al. (2013) is the only one where the atmospheric deposition of silicate has been reported in the western basin. Silicate is essential for diatom skeleton-formation (e.g, Turner et al., 1998). Béthoux et al. (2002) predicted a decrease in this group of phytoplankton in the MS, which dominates the late winter bloom (Estrada, 1996; Gutiérrez-Rodríguez et al., 2010; Marty et al., 2002), as a consequence of the increase in N and phosphorous (P) emissions from the land – through the rivers and the atmosphere – and a decrease in dissolved silicate caused by river dams (Tréguer and De La Rocha, 2013), yielding to a decrease in the Si:N:P ratio. Values of the concentration of these nutrients in the air (usually expressed as $\mu g\ m^{-3}$) are more commonly found in the literature (e.g., Koçak, 2015; Pateraki et al., 2012; Pey et al., 2008; Querol et al., 2001; Viana et al., 2005), and deposition fluxes may be estimated through particle settling velocities (Baker et al., 2010). However, particle settling velocity vary with particle size, aerosol load and meteorological conditions (Gelado-Caballero et al., 2012; López-García et al., 2013), affecting deposition estimates.

In this study we provide data of atmospheric deposition flux of inorganic nutrients and OC in two coastal locations of the northwestern Mediterranean region, an urban area (Barcelona, NE of Spain) and another location with a lower anthropogenic footprint (Blanes, 70 km northeast from Barcelona). For this purpose, a 4.5 y and ca. 3 y time-series were carried out in Barcelona and Blanes, respectively. Given the higher human

impact in Barcelona, we expect to find larger deposition fluxes in Barcelona than in Blanes. Meteorological data as well as registered African intrusions are provided with the aim to explain the variation in the deposition fluxes of the different compounds in both locations. Some considerations about the implication of nutrient deposition on marine planktonic production are discussed.

2.2 Material and methods

2.2.1 Sampling and nutrient determination

Bulk (wet + dry) deposition was collected from May 2011, to December 2015 in Barcelona, with a total of 168 samples. Inorganic nutrients (nitrate, nitrite, ammonium, phosphate and silicate) and total organic carbon (TOC) were analyzed. Samples for inorganic nutrient deposition were collected in Blanes from October 2012, to December 2015, but the 2015 series is incomplete, with a total of 101 samples. Sample collectors were made of 2 l polyethylene cylindrical containers, acid-cleaned and milli-Q rinsed, that were filled with 500 ml of sterile artificial seawater (37 g l^{-1}; NaCl EMSURE, Grade ACS, Merck; Darmstadt, Germany) and left uncovered for two weeks during the cold season − from October to April − and one week during the warm season − from May to September −, to minimize evaporation. Each sampling time, collectors were retrieved and replaced by others that were set at the same position within the device, located on the rooftop of either the Institut de Ciències del Mar at the Barcelona seafront (41.39º N, 2.20º E) or the Centre d'Estudis Avançats in Blanes (41.68º N, 2.80º E).

Two of the polyethylene containers were used for inorganic nutrient analysis, in which mercury chloride was added at 10.5 μg ml^{-1} (final concentration) as biocide. One of

them was left covered during the sampling period and used as control. A third container was acidified with phosphoric acid to pH < 2 and used to determine TOC deposition. The concentration of inorganic nutrients and TOC was measured in the artificial seawater before placing the containers within the device and after the collection. Nutrient samples of 10 ml were immediately frozen at −20 °C until analysis. Inorganic nutrients were measured with an Auto Analyzer AA3 HR (SEAL Analytical; Norderstedt, Germany) following the methods described in Grasshoff et al. (1999). The detection limits in the lowest range (MDL) for the instrument were 0.0100 (NO_3^- + NO_2^-), 0.0015 (NO_2^-), 0.0166 (NH_4^+), 0.0160 (SiO_4^{4-}) and 0.0200 (PO_4^{3-}), all in µmol l^{-1}. For TOC determination, 10 ml samples were collected in pre-combusted glass ampoules (450 ºC, 24 h), acidified with phosphoric acid to pH < 2, heat-sealed and stored at 4 ºC in the dark until analysis. Samples were measured with a TOC-5000 analyzer (SHIMADZU; Kioto, Japan), following the high temperature catalytic oxidation technique described by Cauwet (1994). The system was calibrated daily with a solution of acetanilide.

2.2.2 Meteorological data

Meteorological data of daily rainfall and hourly-wind speed in Barcelona and Blanes was provided by the Spanish Meteorological Agency (AEMET). The concentration of particulate matter in air of size < 10 µm (PM_{10}) was measured every three days at the Institut de Diagnóstico Ambiental y Estudios del Agua in Barcelona, and at La Castanya Biological Station, in the Montseny Mountains (41.76° N, 2.35°E), 50 km to the NW of Blanes. The latter station is considered a background regional air quality site by the EUSAAR network (European Supersites for Atmospheric Aerosol Research), and it is the air quality measurement station closest to Blanes. At La Castanya, a statistical

model provides daily estimates of PM_{10} of African origin ("dust", D, hereafter) (Escudero et al., 2011) that is considered to reach the atmosphere of Barcelona and Blanes at similar concentrations. Therefore, the concentration of PM_{10} from non-Saharan origin (PM_{10NOD}) in both locations was calculated by subtracting the concentration correspondent to dust events (PM_{10D}) from the total PM_{10}.

2.2.3 Characterization of plankton in the water column

In parallel with the atmospheric deposition sampling, we collected monthly samples of chlorophyll *a* (Chl) and prokaryotic (hereafter referred as "bacteria") abundance at the Coastal Monitoring Station of Barcelona (41º 22′ N, 2º 13′ E) from May 2011 to September 2015. In Blanes, we collected samples of chlorophyll, bacterial abundance, and production at the microbial observatory station (41° 40′ N, 2º 48′ E) from November 2012 to November 2014.

For Chl determination, three subsamples of 30 ml of seawater were filtered through Whatman GF/F glass fiber filters and kept frozen at −20 °C. For analysis, filters were immersed in 90% acetone and left in the dark at 4 ºC for 24 h, according to the procedure described in Yentsch and Menzel (1963). The fluorescence of the extract was measured with a calibrated fluorometer (Turner Designs; San Jose, CA, USA).

Bacterial abundance was analyzed following the procedure of Gasol and del Giorgio (2000). Subsamples of 1.8 ml were fixed with 0.18 ml of a 10% paraformaldehyde and 0.5% glutaraldehyde mixture and frozen in liquid nitrogen for at least 30'. Samples were then stored at −80 °C until being analyzed in a Becton Dickinson FACScalibur flow cytometer with a laser emitting at 488 nm (Becton Dickinson; Franklin Lakes, NJ,

USA). For bacteria counting, 400 µl of subsample were stained with a SybrGreen deoxyribonucleic acid fluorochrome and run after 15 min at a low speed (ca. 30 µl min^{-1}). Bacteria were identified on the basis of the fluorescence and light side scatter signatures using the program Paint-A-Gate.

Bacterial production was measured using the [^{3}H] leucine incorporation technique (Kirchman et al., 1985) with the modifications of Smith and Azam (1992). Triplicate aliquots (1.2 ml) of each sample and a trichloroacetic acid (TCA)-killed control were incubated with [^{3}H] leucine at 40-nM (final concentration) for ca. 2 h in the dark. Leucine incorporation was stopped by adding 0.12 ml of TCA (50%) to the live aliquots. Samples were stored at −20 °C until centrifugation and processing, following the procedure of Smith and Azam (1992).

2.2.4 Statistical analyses

The non-parametric Wilcoxon matched pairs test was applied to check for intra-annual variations in meteorological variables and nutrient deposition, and to test if PM_{10} and nutrient deposition statistically varied between locations (considering the same sampling period). To investigate the influence of meteorological factors on nutrient deposition fluxes, a principal component analysis (PCA) was carried out in R (v 3.2.4). Prior to the PCA, data were $\log_{10}$ transformed.

To assess the possible effect of atmospheric deposition on coastal planktonic microorganisms, we carried out cross correlations analyses (by means of the Spearman' ρ coefficient) between the atmospheric deposition data of Barcelona and Blanes, and the concentration of Chl and bacterial abundance measured in the coastal stations of

Barcelona and Blanes, respectively. In Blanes, we also carried out cross correlation analysis between atmospheric deposition and bacterial production.

2.3 Results

2.3.1 Meteorological data dynamics

The inter-annual dynamics of meteorological and air quality variables during the sampling period in Barcelona and Blanes is shown in Fig. 2.1. Daily rainfall presented high variability associated to the seasonal pattern and numerous peaks during the year, with no significant differences between seasons observed in Blanes. Still, in Barcelona, it was significantly higher in fall than in spring ($p = 0.0291$) and summer ($p = 0.0023$) during the study period. Averaged wind speed (WV) followed, on the other hand, a clear seasonal pattern in Barcelona, with significantly higher values in spring and summer than in fall ($p = 0.0002$ and $p < 0.0001$, respectively), and in summer than in winter ($p = 0.0203$). In Blanes instead, WV was significantly higher in fall than in spring and summer ($p = 0.0043$ and $p = 0.0247$, respectively), and in winter than in spring and summer ($p = 0.0012$ and $p = 0.0052$, respectively). With respect to the concentration of PM_{10} in the atmosphere, it was almost double in Barcelona than in Montseny (Fig. 2.1), and attributable to the PM_{10NOD} fraction, that accounted for 95% and 94% in Barcelona and Montseny on average. Both PM_{10} and PM_{10NOD} were significantly higher in Barcelona than in Montseny ($p < 0.0001$). PM_{10} were significantly higher in summer than in fall ($p = 0.0291$) in Barcelona, what must be associated to the dust component, which was significantly higher in spring and summer than in fall ($p = 0.0258$ and $p = 0.0010$, respectively), and in summer than in winter ($p = 0.0005$). In Blanes, PM_{10} were statistically higher in spring and summer than in fall ($p = 0.0498$ and $p < 0.0001$, respectively) and winter ($p = 0.0048$ and $p < 0.0001$,

respectively). PM_{10} were also significantly higher in summer than in spring (p = 0.0018). In this case, the differences seemed to be mainly related to the PM_{10NOD} component that was also statistically higher in summer than in spring (p = 0.0014), fall (p < 0.0001), and winter (p < 0.0001), and in spring than in winter (p = 0.0210).

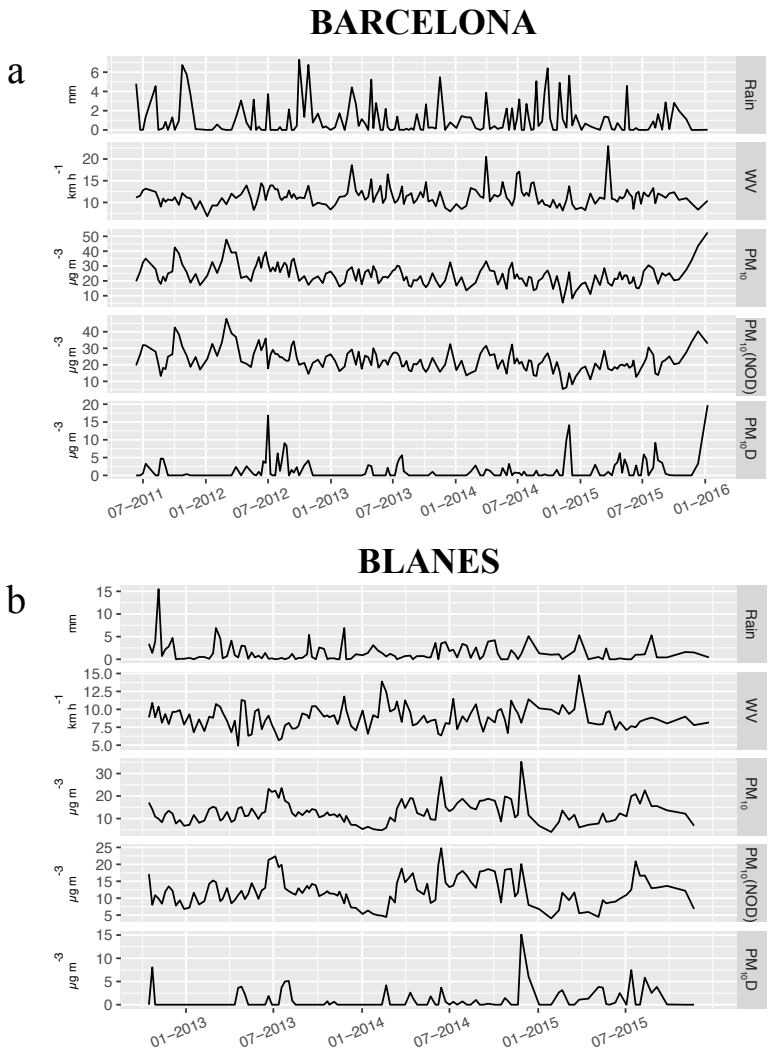

Figure 2.1. Dynamics of meteorological and air quality variables during the sampling period in Barcelona (a) and Blanes – or Montseny in the case of air quality data – (b). Daily data were averaged for each sampling period. Abreviations are as follow: WV = wind velocity; PM_{10} = particulate matter < 10 µm; PM_{10} (NOD) = particulate matter < 10 µm with an atmospheric origin other than dust; $PM_{10}D$ = particulate matter < 10 µm belonging to dust.

2.3.2 Nutrient deposition fluxes

The inter-annual dynamics of nutrient deposition fluxes is shown in Fig. 2.2. In both locations, nitrate (NO_3^-) and nitrite (NO_2^-) daily deposition fluxes followed opposite seasonal patterns (Fig. 2.2). In Barcelona, nitrate deposition was significantly higher during spring and summer than in fall (p = 0.0328 and p = 0.0043 for spring and summer, respectively), or winter (p < 0.0001), and also statistically higher in fall than in winter (p = 0.0014), whereas nitrite was significantly higher in winter and fall than in spring and summer (p < 0.0001) (Fig. 2.3a). In Blanes, nitrate deposition was also significantly higher in spring than in fall and winter (p = 0.0001), and in summer than in fall and winter (p < 0.0001), while nitrite fluxes were significantly larger in spring, fall and winter than in summer (p = 0.0002, p = 0.0001, and p < 0.0001, respectively) (Fig. 2.3b). Deposition of ammonium (NH_4^+) was highly variable, with no significant differences between seasons in any of the locations. The average deposition of NH_4^+ during the study period in Barcelona doubled that in Blanes, and was associated to unusual and very high deposition events. Mean phosphate (PO_4^{3-}) deposition was similar in both locations. It usually remained below the average value of 5 µmol m^{-2} d^{-1}, but very high deposition events (> 50 µmol m^{-2} d^{-1}) were registered in both locations, related with dust intrusions at certain times (Fig. 2.2). In Barcelona, deposition of PO_4^{3-} was the highest in fall and it was significantly larger in fall (p = 0.0369), spring (p = 0.0165), and summer (p = 0.0445), than in winter (Fig. 2.3a). In Blanes instead, PO_4^{3-} deposition was significantly larger in spring than in fall (p = 0.0325) and winter (p = 0.0432) (Fig. 2.3b). The ratio between total inorganic nitrogen (TIN, as the sum of nitrate, nitrite and ammonium) and phosphate (N:P hereafter) was 221 ± 81 in Barcelona and 72 ± 68 in Blanes. The intra-seasonal variability was very high in Barcelona, whereas no significant differences were found among seasons in any of the

locations (Fig. 2.3). In Barcelona, SiO_4^{4-} deposition also doubled that in Blanes, and in both locations was highest during spring (Fig. 2.3). More specifically, in Barcelona, it was significantly higher in spring than in fall (p = 0.0128), and in Blanes in spring (p = 0.0280) and fall (p = 0.0146) than in summer. TOC deposition showed significantly larger fluxes in spring than in summer (p = 0.030) and winter (p = 0.0027) in Barcelona (Fig. 2.3a), while in Blanes no inter-annual differences were found (Fig. 2.3b). The deposition of nitrate, nitrite, ammonium and silicate was significantly larger in Barcelona than in Blanes (p < 0.0001), whereas no statistical differences between the two locations were found for phosphate and TOC deposition.

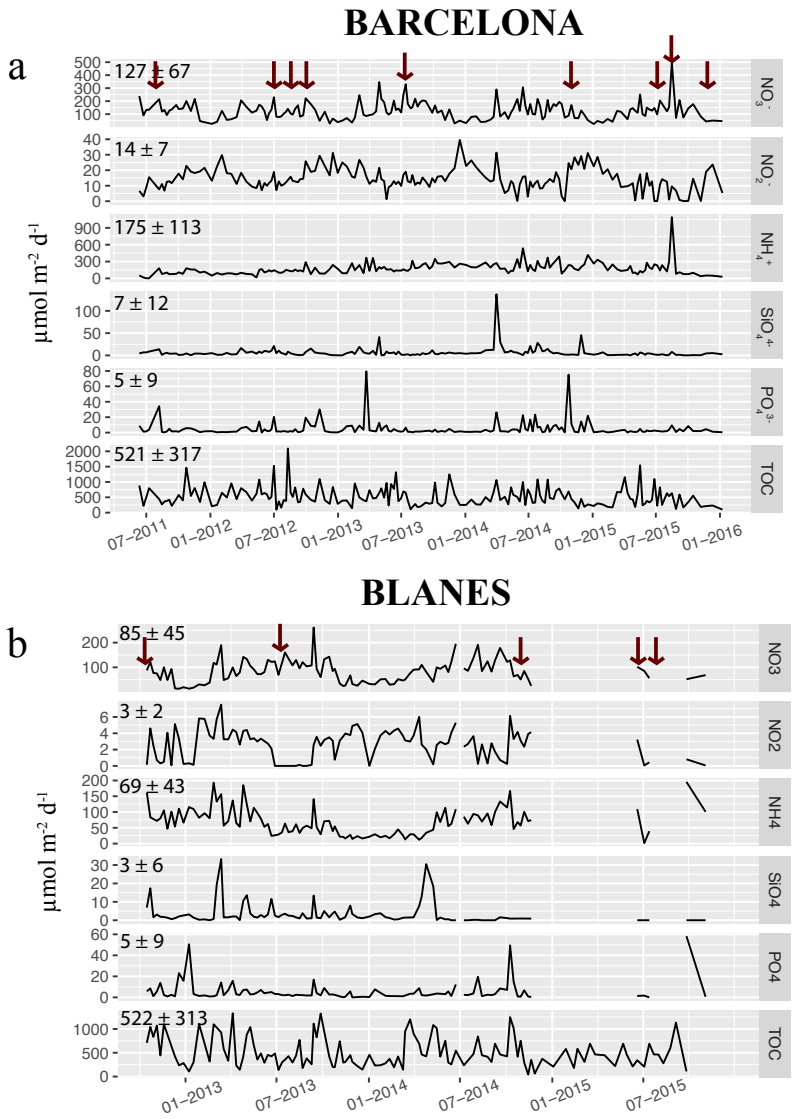

Figure 2.2. Time-series of atmospheric deposition of nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), silicate (SiO_4^{4-}), phosphate (PO_4^{3-}), and TOC in Barcelona (a) and Blanes (b). Average deposition values are indicated in the left up corner for each nutrient. Samples were taken every week during the warm period and every 15 days during the cold period. Red arrows point to times when $PM_{10D} > 5\mu g\ m^{-3}$ (see Fig. 2.1).

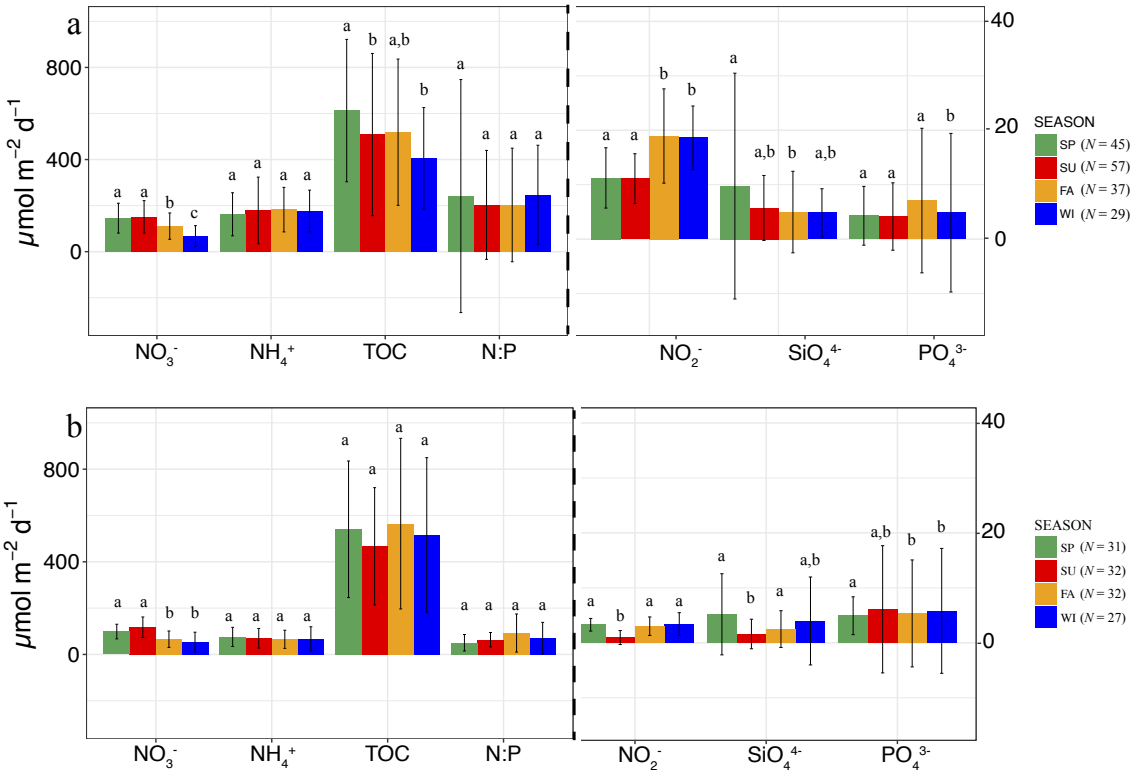

Figure 2.3. Seasonal average of daily deposition of NO_3^-, TOC, NH_4^+, N:P ratio, NO_2^-, SiO_4^{4-}, and PO_4^{3-} in Barcelona (a) and Blanes (b). Note the difference in the scale (on the left part of the plot for NO_3^-, TOC, NH_4^+ and N:P, and on the right part for NO_2^-, SiO_4^{4-} and PO_4^{3-}). Different letters indicate significant differences between season for a given compound. *N* is the number of samples considered per season.

2.3.3 Meteorological variables and atmospheric deposition

PCAs were performed to explain the relationship between nutrient deposition and the average of daily rainfall, WV and PM_{10} (PM_{10D} and PM_{10NOD} were not used for this analyses, as they were highly correlated with total PM_{10}, especially so PM_{10NOD}; see Fig. S2.1 in the Supplementary Material). The first two components (PC1 and PC2) explained 43.5 and 46.7% of the variation in Barcelona and Blanes, respectively (Figs. 2.4a and 2.4b). In general, nutrient deposition was neither correlated with PM_{10} nor with

dust intrusions (Fig. 2.4, red samples), which were related by the second component. Pairwise correlation showed that only NO_3^- was significantly correlated with particle concentration in the atmosphere in Blanes (Fig. S2.1). The first component instead related PO_4^{3-} and SiO_4^{4+} deposition with rainfall, and also NH_4^+ and TOC in Blanes. NO_3^- was orthogonal to this group of variables and PM_{10}, and highly correlated with TOC in Barcelona. NO_2^- and WV showed a higher correlation with the third component, and also NH_4^+ in Barcelona.

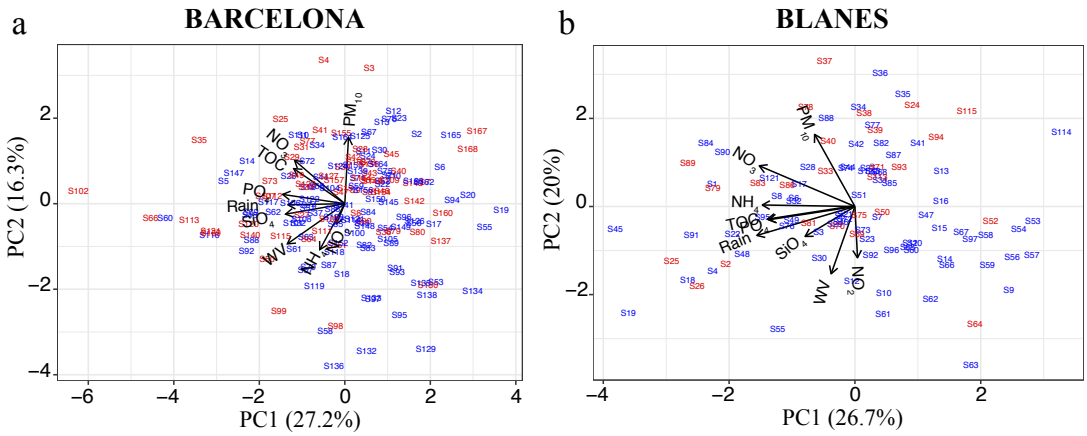

Figure 2.4. Principal component analysis (PCA) showing the relationship between the meteorological data and nutrient atmospheric deposition in Barcelona (a) and Blanes (b). In parenthesis are shown the percentages of variation explained by components one and two. The samples coincident with Saharan dust intrusions in the Iberian Peninsula are painted in red, and in blue are represented the samples not related with dust events.

2.3.4 Impact of atmospheric deposition in the coastal ocean

With the aim to assess the effect of nutrient deposition from the atmosphere on coastal planktonic microorganisms, we performed cross correlations (Spearman) between nutrient deposition and Chl and bacterial abundance measured in the coast of Barcelona and Blanes for a time-lag up to 30 days. In Blanes, we also determined the correlation between atmospheric deposition and heterotrophic bacterial production (HBP). In Barcelona, we found significant and positive correlations between Chl and silicate

during some days for a time-lag < 2 weeks (Fig. 2.5). Heterotrophic bacterial abundance (HBA) was significantly correlated with nitrate for several days after 10 days, and during fewer days with phosphate and TOC after a week (Fig. 2.6). We found instead a significantly negative correlation between HBA and ammonium during the 30 days.

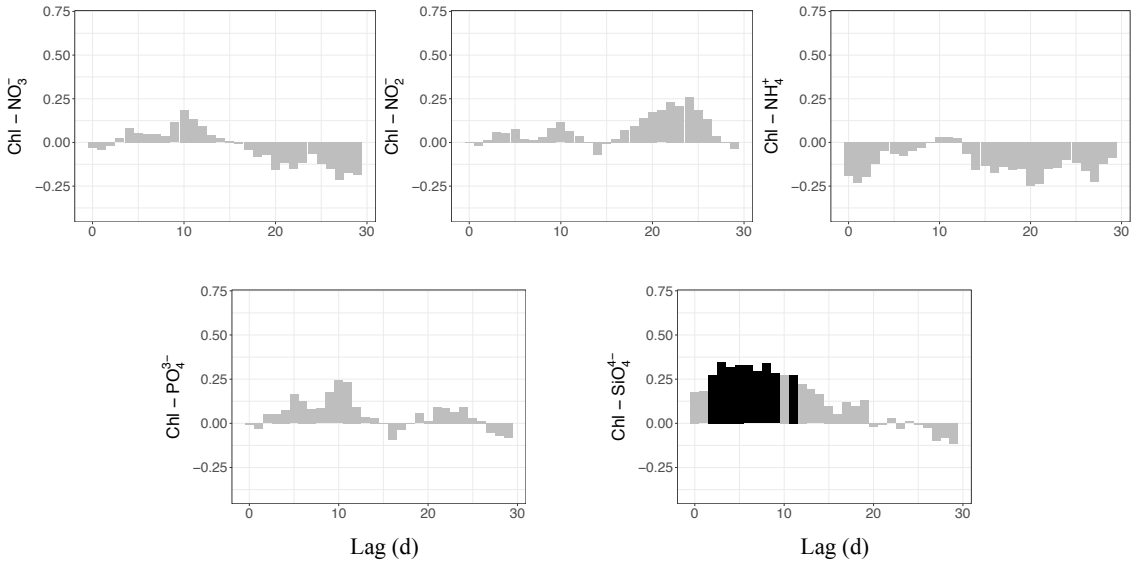

Figure 2.5. Spearman cross correlation between Chl concentration in the coast of Barcelona and atmospheric deposition of NO_3^-, NO_2^-, NH_4^+, PO_4^{3}, and SiO_4^{4-} measured at this location. The correlation was performed for a time-lag up to 30 days. The black bars denote the time-lags in which the Spearman coefficient was significant.

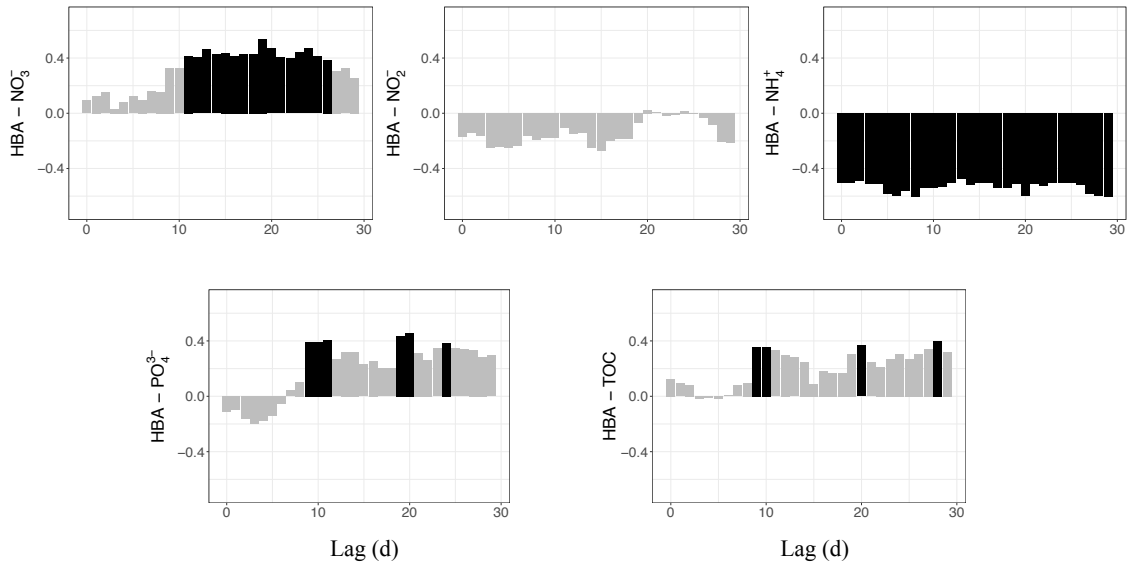

Figure 2.6. Spearman cross correlation between heterotrophic bacterial abundance (HBA) in the coast of Barcelona and atmospheric deposition NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}, and TOC measured at this location. The correlation was performed for a time-lag up to 30 days.

In Blanes, Chl was positively correlated with phosphate and silicate during the first days, while the correlation with nitrite was negative. Instead, after a ca. 3-week time-lag, the correlation turned positive with nitrite for several days, and negative with phosphate and silicate (Fig. 2.7). We did not find significant correlations between Chl and either nitrate or ammonium in any of the locations. HBA was only significantly correlated with nitrate for a time-lag of 13 days (Fig. 2.8), while HBP was significantly correlated with nitrate during almost 30 days and with phosphate initially for a week and for two days after a lag of 2 weeks (Fig. 2.9).

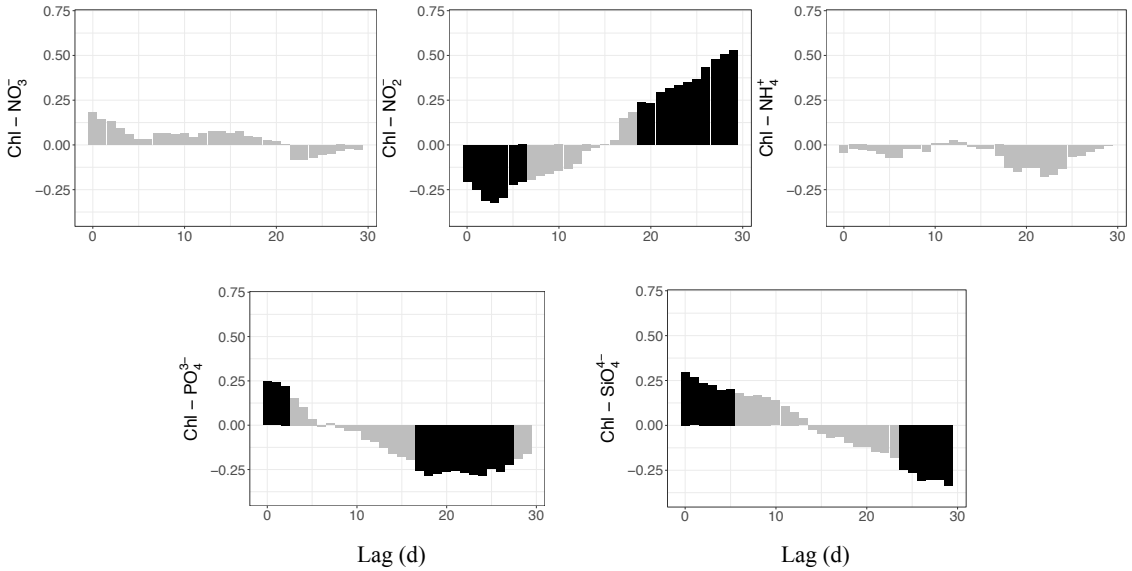

Figure 2.7. Spearman cross correlation between Chl concentration in the coast of Blanes and atmospheric deposition of NO_3^-, NO_2^-, NH_4^+, PO_4^3, and SiO_4^4 measured at this location. The correlation was performed for a time lag up to 30 days. The black bars denote the time-lags in which the Spearman coefficient was significant.

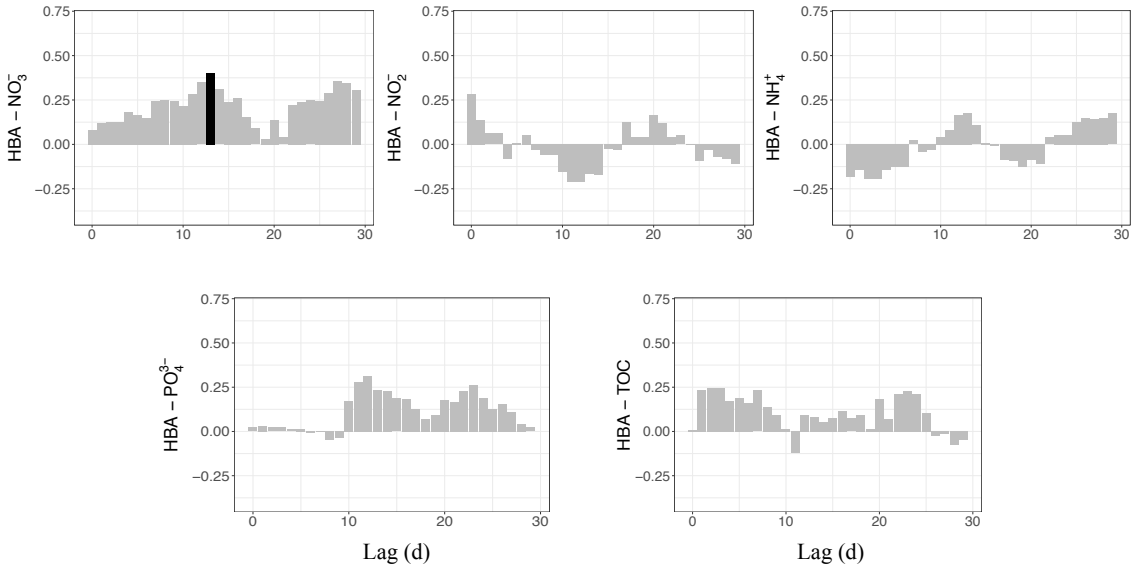

Figure 2.8. Spearman cross correlation between heterotrophic bacterial abundance (HBA) in the coast of Blanes and atmospheric deposition NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}, and TOC measured at this location. The correlation was performed for a time lag up to 30 days.

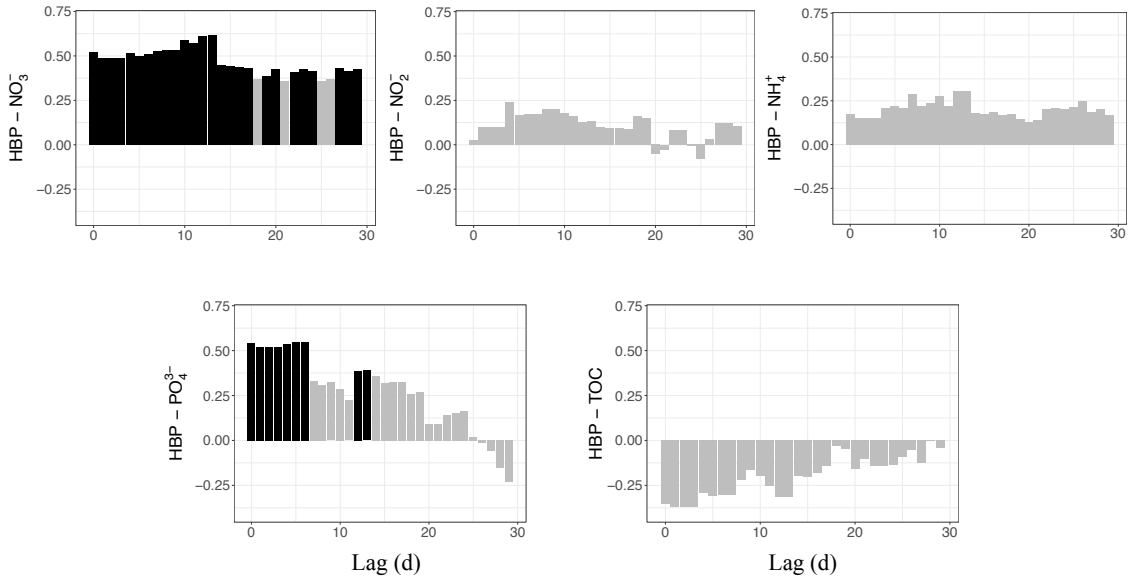

Figure 2.9. Spearman cross correlation between HBP in the coast of Blanes and atmospheric deposition of NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}, and TOC measured at this location. The correlation was performed for a time lag up to 30 days. The black bars denote the time-lags in which the Spearman coefficient was significant.

2.4. Discussion

We have reported data of atmospheric deposition in two coastal locations of the western Mediterranean. Significantly higher deposition fluxes were found in Barcelona than in Blanes for nitrate, nitrite, ammonium and silicate, what must be attributed to the higher

anthropogenic pressure in Barcelona than in Blanes. Among anthropogenic emissions to the atmosphere, vehicle traffic, industrial activity and biomass burning emissions are a continuous source of N and P to the atmosphere in Barcelona (Pey et al., 2008; Querol et al., 2001; Reche et al., 2012). Silicate could derive from gravel and aggregates used in construction, or from sea dredged materials, as the sampling station in Barcelona was closer to the seafront than that in Blanes. This may also be the reason why the deposition of nitrate and TIN in Blanes is within the reported values in remote areas of the Mediterranean basin (Table 2.1), whereas the deposition in Barcelona is much higher. Koçak (2015) determined similar deposition values of nitrate in a coastal rural location in Turkey, but total inorganic nitrogen was still higher in Barcelona (as a consequence of the higher fluxes determined for NH_4^+). It is worthy to note that deposition was not measured directly in all studies, but in some cases it was calculated from the concentration in the atmosphere and the settling velocity of suspended particles (Herut et al., 2002; Koçak, 2015). Furthermore, some studies only measured the deposition from rainwater, not considering the fraction of dry deposition, that can account for more than 50% of nitrate, phosphate and silicate in some cases (Koçak, 2015; Morales-Baquero et al., 2013; Pulido-Villena et al., 2008).

The average deposition fluxes of phosphate and TOC instead were similar in both locations. TOC deposition was similar to that reported by De Vicente et al. (2012) in alpine lakes of Sierra Nevada (SW of Spain), and within those reported by Pulido-Villena et al. (2008) in a remote location of the northwestern Mediterranean (Table 2.1), even though that atmospheric sources of organic carbon would be expected to be higher in polluted urban areas as Barcelona (Pateraki et al., 2012; Viana et al., 2005). The average flux of phosphate determined in this study was similar to that reported by

Duarte et al. (2006) in coastal waters of the north Atlantic ocean. Although atmospheric deposition of total P in the WMS is higher during dust events than during normal weather conditions (Izquierdo et al., 2012; Reche et al., 2009), the bioavaliable fraction of atmospheric P (basically orthophosphate) is higher during non-African events (Christodoulaki et al., 2016; Izquierdo et al., 2012; Nenes et al., 2011). That must be the reason why we found deposition fluxes of P ca. 5 times higher in Barcelona and Blanes than in other (more remote) areas of the Mediterranean. Our values were close to the total (wet+dry) deposition of P measured by Herut et al. (2002), though, what must be related to the more frequent and intense desert dust episodes in the eastern basin (Guerzoni et al., 1999; Krom et al. 2004; Markaki et al., 2003). For the same reason, one could expect a higher deposition of PO_4^{3-} in Barcelona than in Blanes. Hence, the similar flux determined in both locations could be attributed to the higher agricultural (and the concomitant waste burning) practices in the Blanes area, that would counteract for the higher emissions of P from industrial activities and vehicle traffic in Barcelona. The average TIN to phosphate ratio (TIN:P) was therefore much larger in Barcelona (221) than in Blanes (72) (Fig. 2.3), both values within those reported in Mediterranean coastal locations (Markaki et al., 2003, 2010). We registered the highest deposition values of PO_4^{3-} in Barcelona, though, reaching ca. 80 µmol m^{-2} d^{-1} in two occasions, one of them coinciding with a Saharan dust intrusion over the NE Iberian Peninsula (October 2014; Fig. 2.2). The highest deposition flux of PO_4^{3-} measured in Blanes (58 µmol m^{-2} d^{-1}; October 2015) occurred a few days after a dust event, but values of ca. 50 µmol m^{-2} d^{-1} were also registered two other times and were apparently not related to African intrussions, and thus related to European or local sources. The maximum deposition of NH_4^+ (1091 µmol m^{-2} d^{-1}) and NO_3^- (495 µmol m^{-2} d^{-1}) was measured in August of 2015 and coincided with a Saharan dust event that accounted for ca. 25% of

the average PM_{10} during that sampling period (Figs. 2.1 and 2.2). We registered the highest deposition of SiO_4^{4-} in Barcelona during the first days of April of 2014 (137 µmol m^{-2} d^{-1}), probably associated with a Saharan intrusion. PM_{10D} during that sampling time did not exceed the concentration of 5 µg m^{-3} in the atmosphere, but the higher wind velocity could have mobilized the material in the atmosphere, yielding to high deposition fluxes of not only silicate but also NO_3^-, NO_2^-, PO_4^{3-} and TOC (Figs. 2.1 and 2.2). After a few days, we registered one of the highest deposition fluxes of SiO_4^{4-} in Blanes (30.5 µmol m^{-2} d^{-1}; 2.2), while the other nutrients were close to their average value.

With respect to silicate, the average flux determined in Barcelona was slightly higher than those reported by Koçak (2015) and Morales-Baquero et al. (2013) in the eastern and the western MS, respectively. It was somewhat lower in the case of Blanes (Table 2.1). In any case, they were within the values reported by Tréguer et al. (1995), who only measured wet deposition. Furthermore, we determined the same Si:N ratio of 0.02 than Koçak (2015) in both locations. This ratio is much lower than the Redfield ratio of 0.94 proposed for diatoms (Redfield, 1963). Béthoux et al. (2002) observed that, atmospheric inputs of nitrate and phosphate, among other land sources, had increased in the Algero-Provençal basin (WMS) in relation to those of silicate, predicting a relation of 8:21:1 between Si:N:P in 2000. As suggested by these authors, increasing trends in nitrate and phosphate from anthropogenic emissions with respect to silicate may drive the system to a shift from a phytoplankton community dominated by diatoms to assemblages dominated by flagellated microorganisms, including those producing harmful algal blooms. This change has already been observed in other coastal areas such as the Mississippi continental shelf (Turner et al., 1998). We found significant

correlations between atmospheric silicate and chlorophyll measured in the seawater in both locations (Figs. 2.5 and 2.7), which suggests that a reduction in silicate deposition from the atmosphere could actually reduce the proportion of diatoms compared to other phytoplankton groups in Mediterranean coastal waters.

Table 2.1. Comparison of the atmospheric deposition fluxes of NO_3^-, TIN (as the sum of NO_3^- and NH_4^+, and NO_2^- when available), PO_4^{3-}, SiO_4^{4-}, and TOC measured in previous studies and in this study. The superscipts indicates studies where only wet (W) or dry (D) deposition was measured, that were carried out in remote locations (R), or in a coastal area (C). All the studies were performed in the western Mediterranean except those in the eastern Mediterranean (EM).

NO_3^-	TIN	PO_4^{3-}	SiO_4^{4-}	TOC	Reference
-	-	1.1	-	-	Migon et al. (1989)^{R,W}
-	120 - 150	-	-	-	Martin et al. (1989)^{R,W}
-	143	-	-	-	Loÿe-Pilot et al. (1990)^{R}
-	-	-	0-18	-	Tréguer et al. (1995)^{R,W}
55	90	0.8	-	-	Herut et al. (1999)^{EM,W}
96	156	2.7	-	-	Herut et al. (2002)^{EM,D}
-	-	0.05-5.29	-	36-1200	Pulido-Villena et al. (2008)^{R}
-	-	-	-	374	Reche et al. (2009)^{R}
-	91	1.5	-	-	Markaki et al. (2010)^{R}
35	142	-	-	-	Àvila et al. (2010)^{R}
-	-	-	-	540	De Vicente et al. (2012)^{R}
28.8	-	0.5	4.7	-	Morales-Baquero et al. (2013)^{R}
121	190	1.2	4	-	Koçak (2015)^{EM,C}
50	157	5	3	522	Blanes (This study)^{C}
127	316	5	7	521	Barcelona (This study)^{C}

Morales-Baquero et al. (2006; 2013) and Markaki et al. (2010) observed that, while NO_3^- and TIN deposition largely coincided with winter rainfall, the deposition of phosphorous was more variable throughout the year and related with deposition of particulate matter from the Saharan desert, thus mainly associated with dryfall. In contrast, we observed that phosphate showed a higher correlation with rainfall than nitrate, and a low correlation with total PM_{10} and the concentration of particles from the Saharan (Figs. 2.4 and S2.1), while nitrate was significantly correlated with PM_{10}, PM_{10D} and PM_{10NOD} in Blanes (Fig. S2.1). In Barcelona, although nitrate did not show a clear correlation with any of the meteorological parameters, some periods of high deposition appear to coincide with Saharan dust intrusions and high wind speed, all of them being higher during the spring-summer period (Figs. 2.2 and 2.4). One explanation may be the larger size of aerosols containing nitrate compared to phosphate or nitrite, that would favor the deposition of nitrate in the dry form, whereas fine aerosols are expected to deposit mainly in the wet form (Duarte et al., 2006). Some peaks in the deposition of nutrients other than nitrate were also observed coinciding with dust events, though. Silicate was also highly correlated with rain in both locations, while TOC and ammonium were in Blanes only. These results are in agreement with those reported by Koçak (2015), who observed that phosphate, ammonium and silicate deposition were mainly associated with wet deposition in a rural location of southern Turkey. Ridame and Guieu (2002) and Ternon et al. (2010) also suggested that wet deposition was the main source of dissolved P to the WMS. Instead, the deposition of nitrite was more related to wind velocity, especially in Blanes, where both variables were higher during fall and winter.

We observed opposite seasonal patterns for NO_3^- and NO_2^- in both locations, and no seasonal pattern for NH_4^+. As nitrate deposition was always much higher than that of nitrite, this resulted in TIN depositions higher during the summer period. Koçak (2015) observed the same pattern for nitrate, with a winter minimum and a summer maximum, but it must be noted that deposition fluxes were not measured but calculated from particle concentrations in the air, in that study. Querol et al. (2001) instead observed higher concentrations of NO_x during the fall-winter period in the atmosphere of Barcelona, mainly associated to particles of non-mineral origin. One reason might be that atmospheric ammonia – that is higher during summer – reacts with seawater NaCl forming nitrate salts (Viana et al., 2005), thus increasing the NO_3^- inputs in seawater. In any case, the opposite pattern observed for N fluxes with respect atmospheric concentrations, highlights the importance of measuring such fluxes instead of calculating them from atmospheric particle concentrations. In fact, we found a poor correlation between not only nutrient deposition fluxes and PM_{10} (Fig. S2.1), but also with respect the particle air concentration of each of the studied nutrients (R^2 always lower than 0.2). However, it must be considered that atmospheric concentration and deposition were not measured at the same exact location within Barcelona and, in the case of Blanes, atmospheric concentration was in fact measured at another location 40 km away (La Castanya station, Sect. 2.2.2). As a certain spatial variability in atmospheric concentration exists in a given location (e.g., Viana et al., 2005), this must contribute to the high variability observed between atmospheric concentration and deposition.

We showed that the deposition of most of the studied nutrients was correlated either with chlorophyll or with bacterial abundance in Barcelona, and also with bacterial

production in Blanes. Previous studies have focused in the possible impact of atmospherically-derived N and P into the seawater. Koçak (2015) estimated that atmospheric P input might sustain ca. 80 % of the new production in coastal areas of the EMS during the stratification period, and atmospheric N could support 8 times higher new production than that detected for surface waters. We could do a similar approximation for the western basin. Then, converting the average annual deposition fluxes measured in both locations into phytoplankton biomass (considering the Redfiel ratio of 106C:16N:15Si:1P), we would get an annual carbon production of 6.86, 2.72 and 0.13 g C m^{-2} y^{-1} derived from N, P and Si atmospheric fluxes, respectively. The annual new primary production in the western Mediterranean is 18 g C m^{-2} y^{-1} (Béthoux, 1989). Thus, N, P and Si deposition in the western basin would contribute to 38%, 15% and 0.72% of the total new production, respectively. However, we observed that the highest correlation with chlorophyll was found with silicate deposition, a nutrient that remains poorly studied in terms of deposition. Silicate could at certain times fuel diatoms, a group of phytoplankton that needs silica to build its frustules. In addition, we found a high positive correlation between bacterial abundance and nitrate deposition in Barcelona, and to a lesser extent with phosphate and TOC. In Blanes instead, we found non-significant correlations between bacterial abundance and any of the nutrients, but bacterial production was highly correlated with nitrate and phosphate during several days. These results suggest that an increase in the atmospheric deposition of N and P with respect silicate could yield an advantage to the heterotrophic microbial community with respect to the autotrophs. The same approximation used for primary production can be done for bacterial production considering a ratio of 45C:10N:1P (Zweifel et al., 1993). In this case, atmospheric N and P could support 4 – 68% and 1 – 16%, respectively, of the annual bacterial production determined by Gasol et al. (1998)

in a transect offshore Barcelona (20 – 360 mg m^{-2} y^{-1}). The contribution would be higher (> 100%) considering bacterial production measured in more remote locations such as Balearic Islands (Pedrós-Alió et al., 1999). These facts support previous experimental results where the effect of aerosol deposition was found to tilt the balance towards heterotrophy in the MS (Guieu et al., 2014; Herut et al., 2016), although the effect seems to depend on the initial status of the seawater, as well as the concentration, composition and solubility of aerosols into the seawater (Lekunberri et al., 2010; Marañón et al., 2010; Marín et al., 2017). If the current increase of nitrate and phosphate with respect silicate emissions from human activities continues in the future (Béthoux et al., 2002; Christodoulaki et al., 2016; Duce et al., 2008; Migon et al., 2001), the net trophic balance towards heterotrophy could be even greater. Our results thus evidence the importance of assessing the effect of the two main sources of atmospheric deposition in the Mediterranean (i.e. anthropogenic and mineral aerosols) in both the autotrophic and heterotrophic planktonic communities.

5. Conclusions

In this study we provide data of atmospheric deposition fluxes of nutrients in two coastal locations of the western Mediterranean with different anthropogenic footprint, and show that the deposition of inorganic nitrogen and silicate are significantly higher in the location more impacted by the human activity (i.e. Barcelona). A seasonal trend was observed for nitrate and silicate deposition, and less clearly for phosphate and TOC (that depended on the location), with higher deposition values in spring and summer. High deposition fluxes sometimes coincided with Saharan dust outbreaks, which were also more frequent during the dry period. The deposition of phosphate and silicate was mainly associated with rainfall, as was the case for ammonium and TOC deposition in

Blanes. Nitrite deposition instead was mainly associated to wind velocity, which could mean higher sea-spray in the air to react with atmospheric ammonia.

We determined significant correlations between silicate deposition and the concentration of phytoplankton in the seawater in both locations, and bacteria were significantly correlated mainly with nitrate and phosphate deposition. These results point to the importance of the atmosphere as a source of new nutrients into the seawater and that the current increase of N and P emissions from the land with respect to silicate could favor the heterotrophic over the autotrophic communities in Mediterranean coastal waters. The effect of aerosols may be especially strong at certain periods when the concentration of nutrients in the seawater is very low or the deposition is especially high, what tends to occur during the stratification period (from late spring to early fall), coinciding with higher nitrate, phosphate, and silicate depositions.

Acknowledgements

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Supplementary Material

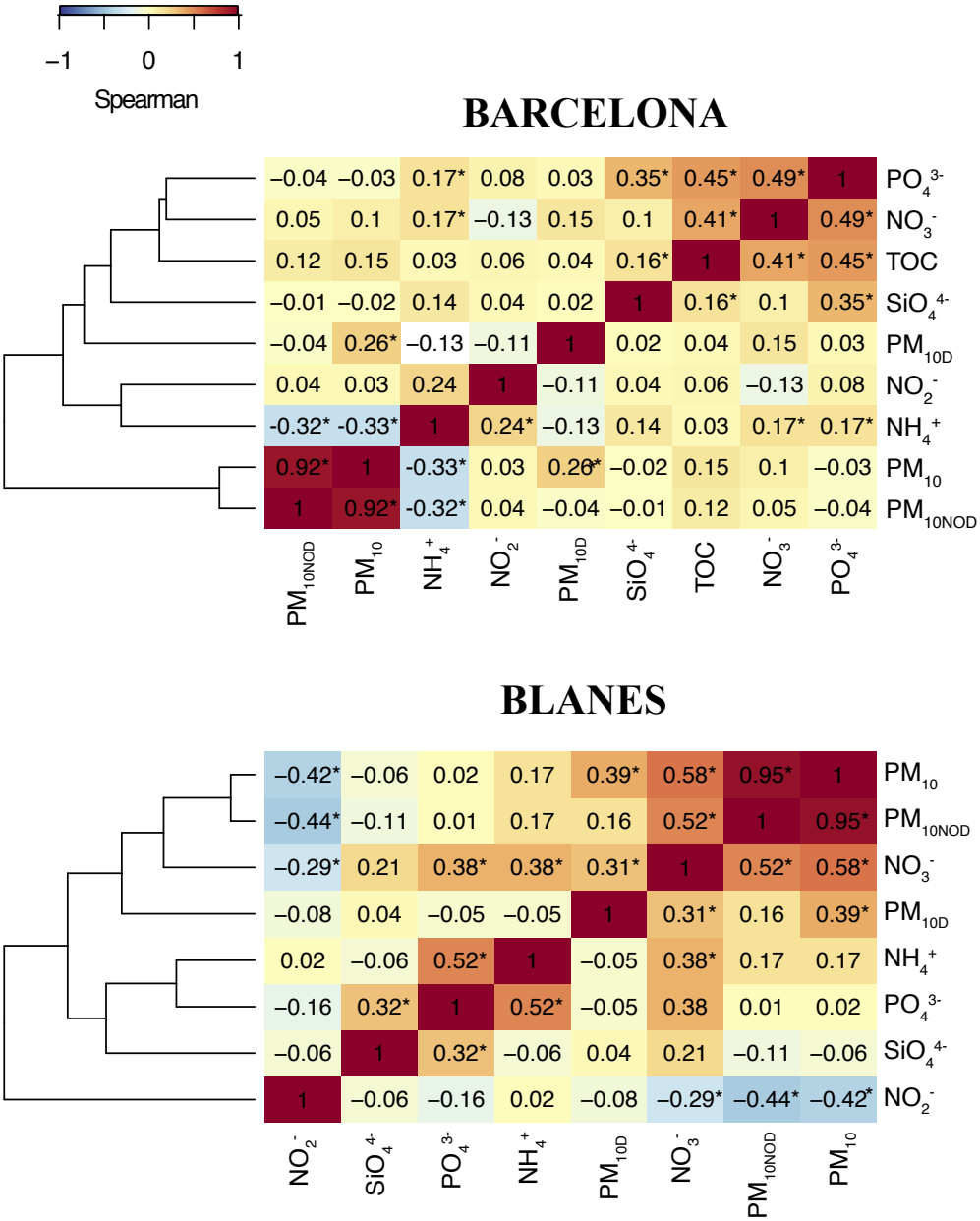

Figure S2.1. Heat map showing the Spearman's ρ coefficient between particulate matter in the atmosphere and nutrient deposition fluxes in both locations. Significant correlations ($p < 0.05$) are indicated with an asterisk (*).

Chapter 3

Anthropogenic versus mineral aerosols in the stimulation of microbial planktonic communities in coastal waters of the northwestern Mediterranean Sea

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Abstract

The atmosphere of the northwestern (NW) Mediterranean Sea is affected by continuous inputs of anthropogenic aerosols and episodic Saharan dust events. These atmospheric inputs deliver to the surface waters high amounts of macronutrients and trace metals that can constitute their main source at certain times of the year. The effect of both anthropogenic and crustal particles over the autotrophic and heterotrophic planktonic community assembles was evaluated through three microcosm experiments carried out in the summer of 2013 and in the winter and spring of 2014 at an urban coastal location of the NW Mediterranean (Barcelona, Spain). Particles were added to seawater at a concentration of 0.8 mg l^{-1}. The results showed that (i) a greater stimulation of the whole community was observed in summer and spring than in winter; (ii) both kinds of aerosols produced an increase in the growth of phytoplankton, although the stimulation of nanoeukaryotes was significantly larger with anthropogenic aerosols; and (iii) bacterial abundance increased more with mineral dust, whereas bacterial production was more stimulated with anthropogenic inputs. Overall, the effect of atmospheric particles was dependent on their composition and solubility in seawater, as well as on the initial biogeochemical conditions present in the seawater and had the potential to change the net metabolic balance of the microbial planktonic community.

3.1 Introduction

The Mediterranean Sea is a low-nutrient low-chlorophyll region containing some of the most oligotrophic waters in the world (e.g., Crise et al., 1999). The atmosphere of the northwestern (NW) Mediterranean Sea is affected by continuous inputs of aerosols from anthropogenic sources and episodic pulses of high magnitude from the Saharan desert (Guerzoni et al., 1999; Pey et al., 2010; Volpe et al., 2009). Roda et al. (1993) and Carratala et al. (1996) estimated annual fluxes of total suspended particles (TSP) of African origin from 1 to 11 g m^{-2} y^{-1} in the eastern coast of Spain. Avila (1997) measured the deposition up to 19 g m^{-2} in a day during a major Saharan dust event at Montseny (NE Spain), and the event of February 2004 in the NW Mediterranean left depositions of more than 22 g m^{-2} (Ternon et al., 2010).

During the stratification period (from the end of spring to early autumn) of the Mediterranean Sea, deposition events of Saharan dust may become the principal source of N, P, Si, Fe, and trace metals on the open surface waters (Carbo et al., 2005; Guerzoni et al., 1999; Markaki et al. 2010; Ridame and Guieu, 2002). On the other hand, sources of anthropogenic aerosols are more continuous but prevail in winter because of anticyclonic conditions (Pey et al., 2010; Viana et al., 2005). Anthropogenic inputs are mainly fueled by the more industrialized NW Mediterranean countries, showing a decreasing north-to-south trend across the basin, in opposition to the decreasing south-to-north Saharan dust gradient (Pey et al., 2010; Volpe et al., 2009). Anthropogenically derived aerosols are characterized by a high load of nitrogen and sulfate species, organic compounds, and trace metals (Dall'Osto et al., 2010; Guerzoni et al., 1999; Pateraki et al., 2012; Querol et al., 2001; Viana et al., 2005). Phosphorous is also common in anthropogenic particles, mainly from chemical fertilizers and

biomass burning in urban incinerators (Migon et al., 2001). High pulses of atmospheric deposition are usually associated with storms (Durrieu de Madron et al., 2011; Ternon et al., 2010); however, dry deposition can also account for an important part of nutrient deposition in the Mediterranean basin. Pulido-Villena et al. (2008a) reported that dry deposition of soluble reactive phosphorous (SRP) represented 79% of the total SRP measured in the southeast of Spain, and in coastal locations of the Mediterranean, the dry deposition of N (Kocak, 2015) and trace metals (Durrieu de Madron et al., 2011) has been reported to be equal or higher than wet deposition. Furthermore, current climate change models predict a mean reduction of precipitation by about 20% (IPCC, 2008), and consequently, a dryer and warmer climate in the Mediterranean region is expected (Durrieu de Madron et al., 2011, and references therein). This is likely to produce an increase in dry versus wet deposition in the basin.

In the NW Mediterranean, although P is generally considered the main limiting nutrient throughout the year (Marty et al., 2002), given the low concentrations of both N and P during the stratification period in the surface waters of the Mediterranean Sea, both nutrients can actually co-limit microorganism growth at this time of the year in either coastal (Guadayol et al., 2009) or open waters (Moutin et al., 2012). Therefore, the N and P content of the atmospheric particles leads to the hypothesis that the effect of aerosols (independently of their origin) in the NW Mediterranean would be higher during the stratification season. This seems to be especially true for coastal waters, where, furthermore, discharges from rivers are also lower during the dry season (from May to November). Terrestrial inputs through major rivers are usually the principal source of macro-nutrients to coastal waters of the NW Mediterranean, but atmospheric inputs may dominate the nutrient supply at certain times (Durrieu de Madron et al.,

2011; Guieu et al., 2010a; Migon et al. 2001). Martin et al. (1989) estimated that at the annual scale, dust inputs were of the same order of magnitude as the downstream flow of rivers discharging to the western Mediterranean and that atmospheric fluxes of Cu, Pb, and Cd exceeded the river input by one to two orders of magnitude. On the other hand, Ludwig et al. (2009) found an overall decrease in the riverine freshwater discharge in the Mediterranean region, and Romero et al. (2013) determined a significant and moderate decrease in P and N loads in Mediterranean rivers, respectively, mainly due to the ban of phosphates in household detergents and the technical improvements in wastewater treatments plants. Atmospheric N and P inputs from anthropogenic sources and forest fires associated with droughts are predicted to increase in the Mediterranean basin (Durrieu de Madron et al., 2011). In coastal areas, furthermore, anthropogenic atmospheric fluxes are expected to increase rapidly because of the demographic pressure (Lionello et al., 2006). More specifically, Paerl et al. (1995) estimated that the delivery of atmospheric N to coastal regions in Europe had increased by 50–200% during the past 50 years, while P delivered to the atmosphere has been calculated to reach 0.55×10^{9} mol $year^{-1}$ in treatment plants from urban coastal locations of the NW Mediterranean (Migon et al. 2001).

Barcelona coastal area receives the discharge of the Besòs River to the north and the Llobregat River to the south, which have a typical Mediterranean river regime with a relatively low mean water discharge and extreme seasonal variations (Liquete et al., 2010). The catchment area between the two rivers houses over 4.6 million people (Romero et al., 2014), and it is consequently influenced by numerous human activities. Still, very low nutrient concentration can temporally be found in Barcelona coastal waters. Arin et al. (2013) reported minimum values of total inorganic nitrogen (TIN) as

low as 0.04 μM and <0.01 μM for phosphate during summer and autumn, respectively, and very low values were also reported at certain times during winter (minimum values of 0.86 and 0.06 μM for DIN and phosphate, respectively). Hence, even at times when nutrient concentrations are expected to be relatively high in the water, low concentrations and strong imbalances may be observed in Mediterranean coastal waters, opening windows of opportunity for the nutrients delivered from atmospheric deposition to have an impact on plankton production, as pointed by Gallisai et al. (2014). For example, although carbon (C) is believed to be in excess with respect to phosphorous (P) and nitrogen (N) (Marty et al. 2002) in the whole basin, it has been observed that dissolved organic carbon may limit or co-limit bacterial growth at certain times of the year either in open (Thingstad et al., 1997) or coastal waters (Pinhassi et al., 2006) of the NW Mediterranean. Thus, the organic matter supplied by aerosols (especially from anthropogenic sources) could stimulate bacterial production in these conditions. Considering the special characteristics of coastal (urban) waters, it is therefore of interest to evaluate the effect of atmospheric particles on the marine community at different initial conditions, considering the concentration of nutrients in the seawater and the different composition of the atmospheric particles.

Previous studies have reported that nutrients and metals delivered from atmospheric sources may ultimately have an impact on autotrophic and heterotrophic planktonic production in marine surface waters (e.g., Herut et al., 2005; Marañón et al., 2010; Romero et al., 2011). A problem still not solved, though, is the question of whether a stimulation of heterotrophic bacteria (HB) is directly produced by nutrients delivered from atmospheric inputs or whether there is a mediated effect through the phytoplankton response. In some of the previous amendment experiments carried out

with Saharan dust in the (either in open or coastal waters) Mediterranean Sea, a stimulation in bacterial respiration and growth was the first observed response (Lekunberri et al., 2010). In contrast, other experiments showed a quicker response of the autotrophic community (Herut et al., 2005; Laghdass et al., 2011). The overall outcome could depend on the degree of oligotrophy of the region, as suggested by Marañón et al. (2010). Regarding anthropogenic aerosols, a few works performed in the open Mediterranean Sea during summer have reported a more positive effect on chlorophyll stimulation than Saharan particles, while no appreciable effects were found on bacterial abundance (Bonnet et al., 2005; Ternon et al., 2011); however, no comparable studies have been performed at other times of the year.

Aerosol particles are also a major source of trace metals in seawater. This is especially true for anthropogenic aerosols, which in addition are much more soluble (e.g., Durrieu de Madron et al., 2011; Jordi et al. 2012). Some metals are essential in trace amounts for plankton physiology but can be toxic at higher concentrations. Experimental studies have already shown the negative effect of copper on phytoplankton cells (Mann et al., 2002; Paytan et al., 2009; Sunda and Huntsman, 1998). In the Mediterranean Sea, previous studies based on satellite data have also suggested a negative correlation between Cu from atmospheric deposition and chlorophyll *a* (Gallisai et al., 2014; Jordi et al., 2012), but this has not been experimentally tested yet. Despite the fact that cyanobacteria have been reported to be among the most sensitive species to copper toxicity in the laboratory (Mann et al., 2002), no attempts have been made to evaluate the toxicity of dissolved copper from aerosols on marine cyanobacteria in the NW Mediterranean coastal waters.

The main aim of this study is to evaluate the effect of mineral and anthropogenic aerosols (dry particles) on marine planktonic assemblages at different times of the year in an urban coastal location of the NW Mediterranean. On the basis of previous knowledge, we hypothesize that (i) both autotrophic and heterotrophic communities will show a positive response to both kinds of aerosols in (late) spring and summer, given the stratification conditions, whereas no or weaker effects are expected during winter because of higher basal nutrient concentrations and (ii) HB will be more stimulated with aerosols of anthropogenic origin, given their higher content in organic carbon. We also aim to test the toxicity of dissolved Cu supplied by the aerosols on cyanobacteria.

3. 2 Materials and Methods

3.2.1 Aerosol collection

TSP were collected in the summer of 2013 and in the winter of 2013–2014 on rectangular quartz fiber filters (203×254 mm, Munktell) with a MCV CAV-A/mb high volume sampler operating at a flow rate of 30 m^3 h^{-1} for 24 h. The device was placed on the rooftop of the Institut de Ciències del Mar, located at the Barcelona seafront (41º 23′ 08″ N, 2º 11′ 46″ E). Saharan dust was collected on the basis of advanced event warnings from the website www.calima.ws. Anthropogenic aerosols were collected at all other times.

Particulate matter trapped in the collected filters was gravimetrically determined, and the filters were subsequently cut into two equal sections. A half of each filter was kept at 4 ºC and employed to characterize the chemical composition of the particles (the specific methodology is detailed in the Supplementary material and can be found elsewhere, e.g. Moreno et al., 2006; Querol et al., 2001). Once the chemical analyses

were completed, the filters were ultimately assigned as Saharan or anthropogenic categories according to the element ratios determined by Guieu and Thomas (1996), Guieu et al. (2002), Nava et al. (2012), and references therein (see Supplementary methods). The second half of the filter was frozen at −20 ºC until used for the amendment experiments (less than 3 months since filter collection). Particles were extracted from the filters into 250 ml of artificial seawater (NaCl EMSURE, Grade ACS, MERCK) by sonication for 20 min (7 kHz) in a Bandelin SONOREX Digital 10 P Ultrasonic bath. This solution was used to inoculate the microcosm stimulation experiments.

3.2.2 Microcosm experimental design

Microcosm experiments were carried out in the late summer of 2013 and in the winter and spring of 2014. Surface water was collected at the Coastal Monitoring Station of Barcelona (41º 22′ 33″ N, 2º 12′ 58″ E, 40 m depth) using acid-cleaned carboys and transferred to the laboratory in less than 1 hour. The water was filtered in situ through a 150-µm nylon mesh to remove macrozooplankton. During the spring experiment, samples for trace metal determination were also collected. In this case, all the material used was cleaned following trace-metal cleaning procedures detailed by Bruland and Franks (1979). Once in the laboratory, seawater from the carboys was randomly distributed into acid-cleaned methacrylate containers and filled up to 15 l. Microcosms were incubated in a temperature-controlled chamber at the in situ surface water temperature and the corresponding photoperiod at that time of the year (Table 3.1). Measured light intensity inside the experimental containers was approximately 225 µmol photons m^{-2} s^{-1}, which is around the saturating irradiance reported in the Catalan coast for most of the year (Guadayol et al., 2009). An initial sample was taken to

determine the biogeochemical conditions of the seawater on the sampling day (hereafter referred to as T_0). The concentrations of nitrate (NO_3^-), phosphate (PO_4^{3-}), chlorophyll *a* (Chl *a*), and heterotrophic bacterial abundance (HBA) measured at T_0 were compared with measurements from the 10-year time series of the Coastal Monitoring Station of Barcelona to contextualize the observed microbial response given the initial biogeochemical conditions.

Roughly 24 h later, a ca. 500-ml subsample of each microcosm was taken to analyze the most sensitive parameters (inorganic nutrients, total organic carbon (TOC), and heterotrophic bacterial production (HBP)), and aerosols were subsequently added. In the summer experiment, two microcosms were amended with Saharan dust (S) and another two with aerosols of anthropogenic origin (A) to a final concentration of 0.8 mg l^{-1} in both cases. The remaining two microcosms were left unamended, serving as controls (C1). In the spring experiment, the conditions were the same, but two more microcosms were added as a second control (C2). In C2, a blank filter was sonicated in the same way as atmospheric filters, and the same solution volume added to A and S microcosms to reach the final concentration of 0.8 mg l^{-1} was also added to the C2 containers to observe the possible effects attributed to the filters themselves. Because of the higher frequency of pollution episodes of anthropogenic origin from Europe during winter, we decided to have two anthropogenic aerosol treatments of different chemical composition (A1 and A2) in the winter experiment. C1 and C2 controls were also used. Table S3.1 in the Supplementary material summarizes the design of the three experiments. Once the aerosols were added, all microcosms were gently stirred with a sterile pipette, and samples for all the parameters were taken about 1 h after the amendment. Considering the deposition fluxes reached during Saharan events previously reported in the NW

Mediterranean, the concentration of 0.8 mg l^{-1} is equivalent to a dust event of medium–high intensity of 8 g m^{-2} into a mixed layer of 10 m depth, which is the approximate depth of the thermocline during the stratification period in the NW Mediterranean (D'Ortenzio et al., 2005). Although the same deposition rates do not currently occur for anthropogenic particles, we decided to use the same concentration for the anthropogenic microcosms to compare the effect of both types of aerosols on marine planktonic microorganisms at the same concentration. Furthermore, with the current trend of increasing anthropogenic emissions at the Mediterranean and global scales (Reche et al., 2009), and the prediction of more prevalent winds from Europe over the Mediterranean (McInnes et al., 2011), this concentration might actually be reached in a short-term temporal scale.

Samples were taken from each container by siphoning water through autoclaved, milli-Q rinsed glass tubes. Sampling for most of the parameters was performed daily for 5 days after the addition of aerosols in the winter experiment and for 4 days during summer and spring. The biogeochemical variables measured in all the microcosms were inorganic nutrients, TOC, Chl *a*, bacterioplankton, and pico- and nanophytoplankton (Table S3.1). Samples for HBP, heterotrophic nanoflagellate (HNF) determination, and particulate organic carbon (POC), instead, were only taken from one of the controls, C1 in summer and C2 in winter and spring. All the variables were measured daily except for POC samples, which were collected at the beginning (T_0) and at the end of each experiment. In addition, samples for total dissolved copper (TDCu) analysis were taken during the spring experiment to check the possible toxic effect of atmospheric Cu on cyanobacteria. These samples were collected at T_0, after the aerosol addition and at the

end of the experiment in C2, A, and S microcosms. Before the last sampling day, approximately two-thirds of the volume still remained in the microcosms.

3.2.3 Analytical procedures

Inorganic nutrients (nitrate, nitrite, ammonium, and phosphate) were analyzed following the methods described in Grasshoff et al. (1999). Samples of 10 ml were kept frozen at −20 °C until measurement with a SEAL Auto Analyzer AA3 HR. The detection limits for the instrument were 0.0060 (nitrate + nitrite), 0.0015 (nitrite), 0.0003 (ammonium), and 0.0200 (phosphate), all in μmol l^{-1}. The coefficient of variation (CV) of 10 replicates was 0.20% for all the nutrients.

For TOC determination, 10 ml samples were collected in pre-combusted glass ampoules (450 ºC, 24 h), acidified with phosphoric acid to pH 2, heat-sealed, and stored in the dark until examined. Samples were measured with a SHIMADZU TOC-5000 analyzer, following the high-temperature catalytic oxidation (HTCO) technique (Cauwet, 1994). The system was calibrated daily with a solution of acetanilide. The detection limit of the instrument was 5 ppb of C and the CV (from 128 samples) was 1.44 ± 0.76%.

The total Chl *a* was determined by filtering 30 ml of triplicate subsamples through Whatman GF/F glass fiber filters that were kept frozen at −20 °C until analysis. For analysis, filters were immersed in 90% acetone and left in the dark at 4 ºC for 24 h, according to the procedure described in Yentsch and Menzel (1963). The fluorescence of the extract was measured with a calibrated Turner Designs fluorometer. The averaged CV (three replicates from 256 samples) was 4.41 ± 4.35%.

Subsamples of 1.8 ml were fixed with 0.18 ml of a 10% paraformaldehyde and 0.5% glutaraldehyde mixture for the determination of HBA and autotrophic pico- and nanoplankton by flow cytometry using a Becton Dickinson FACScalibur flow cytometer with a laser emitting at 488 nm (Gasol and del Giorgio, 2000). Samples were stored at −80 °C until analysis. For bacteria counting, 400 µl of subsample were stained with a SybrGreen deoxyribonucleic acid fluorochrome and run after 15 min at a low speed (ca. 30 µl min^{-1}). Subsamples of 800 µl were run at a high speed (ca. 80 µl min^{-1}) for pico- and nanoplankton determination. These groups were identified on the basis of their fluorescence and light side scatter signatures. The CV of the measurements was 2% for HBA and 2.5% for phytoplankton.

HBP was measured using the [^{3}H] leucine incorporation technique (Kirchman et al., 1985) with the modifications of Smith and Azam (1992). For each sample, triplicate aliquots (1.2 ml) and a trichloroacetic acid (TCA)-killed control were incubated with [^{3}H] leucine (40-nM final concentration, 108 Ci $mmol^{-1}$) for ca. 2 h in the dark at the experimental temperature. Leucine (Leu) incorporation was stopped by adding 0.12 ml of TCA (50%) to the live aliquots. Samples were stored at −20 °C until centrifugation and processing, following the procedure of Smith and Azam (1992). Conversion of Leu incorporation rate to bacterial production was done assuming a 0.073% of Leu in protein and a C:P of 0.86 (Simon and Azam, 1989), using an isotope dilution factor of 2, typical for oligotrophic water, and bacterial cell volumes determined by flow cytometry. The averaged CV (three replicates from 129 samples) was 6.92 ± 5.85%.

HNF abundance was estimated by epifluorescence microscopy after fixing a 50-ml subsample with glutaraldehyde at 1% (final concentration). A subsample of 30 ml was

stained with DAPI (4', 6'diamidino-2-phenylindole) at 5 µg ml^{-1} final concentration following the procedure of Porter and Feig (1980) and filtered through 0.8-µm black polycarbonate membranes. The filters were then mounted on microscope slides and kept frozen at −20 ºC. Counts were done on an Olympus DP72 epifluorescence microscope at 1000X magnification. Between 200 and 400 nanoflagellates were counted on each filter, and they were classified in four size classes (<5 µm, 5–10 µm, 10–20 µm, >20 µm) using a calibrated ocular micrometer. Autotrophic organisms were distinguished from the heterotrophic ones by the red fluorescence of chlorophyll under blue light excitation.

For POC analyses, 1000 ml of sample were filtered through pre-combusted (450 °C, 4 h) glass fiber filters (Whatman GF/F). The filters were dehydrated for 24 h and then kept at −80 ºC until analysis. Before measurements in a PerkinElmer 2400 CHN analyzer, all the filters were thawed in an HCl-saturated atmosphere for 24 h to remove inorganic compounds and dehydrated again for 24 h.

Samples of 100 ml were filtered through 0.2-µm polycarbonate filters (Durapore, Millipore) for TDCu determination. They were acidified to pH < 2 with HCl (30%) Suprapur quality (Merck, Millipore) directly after collection and kept at room temperature until analysis. Subsamples of 20 ml were UV-irradiated for 4 h and analyzed by cathodic stripping voltammetry following the methodology described by Campos and van den Berg (1994). A µAutolab (Metrohm) voltameter with a Metrohm VA 663 static mercury drop electrode was used. Then 50 µl of the ligand salicylaldoxime and 200 µl of the buffer EPPS (1M) were added until reaching a final pH of 8.0–8.5. An internal standard of copper (20 µM; pH=2) was added in doses of 20

µl with a Metrohm 800 dosino burette until a concentration of 0.1 µM was reached in the sample. The CV from six replicates was 0.55%.

In general, all the samples were measured in less than 2 months after the experiments were carried out with the exception of TDCu samples, which were measured 5 months later.

3.2.4 Biomass estimation

The concentration of phytoplankton, HBA, and HNF was transformed into biomass values with the aim of determining the distribution of living POC inside the microcosms. Chl *a* values were converted to carbon using a factor of 50 µg C per µg of Chl, a value within the range reported by Delgado et al. (1992) for surface waters of the NW Mediterranean. Bacterial biomass was estimated using a carbon conversion factor of 0.35 pg C μm^{-3} (Bjørnsen, 1986). The cell volume of HNFs was established from the mean value of each size class, assuming a prolate spheroid shape (Romero et al., 2011). Conversion to carbon was then calculated with the equation used by Verity et al. (1992) (Eq. 3.1).

$$pgC\ cell^{-1} = 0.433 \cdot (\mu m^3)^{0.863} \qquad \text{Eq. 3.1}$$

3.2.5 Statistical analyses

An analysis of covariance (ANCOVA) was applied with all the data of the biogeochemical variables measured in both controls (C1 and C2) during the winter and spring experiments to check if there were significant differences between them. The factors were the CONTROL-TYPE (C1 or C2), the EXPERIMENT (winter or spring),

and the incubation TIME. TIME was used as a continuous factor (covariate), and a knotted spline effect was applied with 5 knots (Stone and Koo, 1986). As results showed no significant differences for any of the biogeochemical variables measured in both controls (Table S3.2), we refer to all the results with respect to C1 in summer and the average of both controls in winter and spring. Therefore, from now on, all the controls will be referred to as C.

The relative increase in a particular biogeochemical variable in each microcosm was calculated through the after–before ratio (ABR), described in equation 3.2.

$$ABR_X = \frac{X_F}{X_I} \qquad \text{Eq. 3.2}$$

where X_F is the maximum value reached for a given biological variable (Chl *a*, *Synechococcus*, picoeukaryotes, nanoeukaryotes, HBA, HBP, and HNF) in a given time after the addition and X_I is the value of the given variable before the addition. As shown in previous abiotic assays with Saharan dust, inorganic nutrients are likely released during the first few hours (Ridame and Guieu, 2002; Romero et al., 2011). Hence, for total inorganic nitrogen ((TIN), hereafter referred to as the sum of nitrate, nitrite, and ammonium), total inorganic phosphorous ((TIP), basically orthophosphate), and TOC, X_F is the value measured in the time just after the amendment. Then, the aerosol-induced ratio (AIR) was calculated to compare the increase observed in the aerosol-amended microcosms with that in the controls (Eq. 3.3).

$$AIR_{X(aerosol,control)} = \frac{ABR_{X(aerosol)}}{ABR_{X(control)}} \qquad \text{Eq. 3.3}$$

where aerosol refers to A or S. Then an AIR value equal to 1 indicates that there is neither increase nor decrease for a given variable in the aerosol-amended containers (A, S) with respect to the controls (C), whereas an AIR above or below 1 points to a positive or negative stimulation in the amended containers, respectively.

A two-way ANOVA (ANOVA1) was then applied considering all AIR data determined from the three experiments to check if there were significant differences in the response between the two types of aerosol and between seasons. As the chemical composition of the particles collected from the atmosphere on a given day may not always be representative of the average supply at a given season, a further two-way ANOVA (ANOVA2) was performed using as dependent variables the AIR values of the biological variables divided by TIN and TIP ratios (AIR_{TIN} and AIR_{TIP}) to compare the relative increase of the biological variables related to nutrient addition. To simplify, these ratios were expressed as the name of the biological variable followed by a slash ("/") and TIN or TIP. AIR_{HBA} and AIR_{HBP} were also divided by AIR_{TOC} and AIR_{Chl} to determine whether bacteria rather take up the organic carbon from the atmospheric particles or from the autotrophic community. When significant differences were found between seasons, a post-hoc Tukey HSD test was applied to determine which levels were different. Statistical analyses were performed with JMP (SAS) version 10.0. Homoscedasticity of variances was checked for AIR values, and they were homogeneous for almost all the variables. Otherwise, Welch's test was applied, and the Dunn method with the Bonferroni adjustment was used to determine the differences between seasons. Significance was considered for $p<0.05$.

3.3 Results

3.3.1 Aerosol composition

The chemical composition of the collected filters can be found in the Supplementary material (Tables S3.3 and S3.4). Saharan dust was mainly composed of silica (32 ± 6% of TSP on average) and aluminum (11 ± 2%), whereas the principal component of the anthropogenic aerosols was organic carbon (16 ± 5%). Nitrate and ammonium reached higher percentages in the anthropogenic filters (7.52 ± 3.26% and 1.53 ± 1.32%, respectively) than in the Saharan (2.95 ± 1.19% and 0.39 ± 0.19%, respectively). Phosphorous contributed in a similar percentage to both kinds of aerosols, 0.11 ± 0.02% in the anthropogenic and 0.10 ± 0.04% in the Saharan. Anthropogenic aerosols always presented a N:P ratio quite above Redfield's (from 24 in summer to 124 in winter), while Saharan dust presented slightly lower ratios, 25 in summer and 27 in spring.

3.3.2 Initial conditions

Biogeochemical conditions determined at the beginning of the three experiments are shown in Table 3.1. Nitrate, TIP, Chl *a*, and HBA initial concentrations are compared with data from the 10-year time series of the Coastal Monitoring Station (Fig. 3.1). In the summer experiment, NO_3^- concentration was above that predicted for that day of the year (DOY) (Fig. 3.1a), whereas TIP was below (Fig. 3.1b). A concentration of nitrate much higher than the seasonal average was also determined during the monthly sampling carried out at this station 6 days earlier. This larger concentration is probably associated with a discharge from the Besòs River, as 17 mm of accumulated rainfall were registered during the 10 days before the sampling date (data provided by the

Spanish Meteorological Agency), whereas no anomalous atmospheric deposition events were registered. This drove the system to a high TIN:TIP molar ratio (hereafter N:P) of 46. HBA, and especially Chl *a*, had a concentration below average conditions at that time of the year (Figs. 3.1c and 3.1d). During the winter experiment, initial concentrations of both inorganic N and P were low compared with those predicted and Chl *a* concentration was slightly higher than expected, pointing to a post-bloom situation. The previous phytoplankton bloom could have been induced by a dust event registered on February 18 (www.calima.ws), 1 week before the start of this experiment. The initial concentrations of NO_3^- and TIP measured in spring were slightly higher than those predicted. In this case, both Chl *a* and HBA were very close to the expected concentration at that DOY. On the other hand, the concentration of TOC was very similar at the beginning of all the experiments, and the C:N:P ratio was always well above the Redfield ratio of 106:16:1 (Redfield, 1934) (Table 1). POC initially present in the seawater was much higher in winter and spring than in summer, in good agreement with the higher values of Chl *a*.

Table 3.1. Biogeochemical conditions determined at the beginning of the three experiments (T_0) in the surface waters of the Barcelona coast. SST= sea surface temperature. n.d.: not determined.

	SUMMER	**WINTER**	**SPRING**
Date	**18.09.2013**	**25.02.2014**	**12.05.2014**
SST (ºC)	23.4	13.3	17.6
Photoperiod (light h)	12.5	11.0	14.5
NO_3^- (µM)	1.82	1.32	1.71
NO_2^- (µM)	0.11	0.37	0.84
NH_4^+ (µM)	0.35	0.57	1.85
PO_4^{3-} (µM)	0.05	0.05	0.26
Chl *a* (µg l^{-1})	0.44	1.90	1.22
HBA ($\times 10^6$ cell ml^{-1})	0.66	0.51	1.15
HBP (µg C l^{-1} d^{-1})	1.91	4.53	1.19

Synechococcus ($\times 10^3$ cell ml^{-1})	44.97	6.77	41.49
Prochlorococcus ($\times 10^3$ cell ml^{-1})	7.30	2.86	3.33
Picoeukaryotes ($\times 10^3$ cell ml^{-1})	6.15	3.77	1.77
Nanoeukaryotes ($\times 10^3$ cell ml^{-1})	2.90	1.59	3.73
HNF ($\times 10^3$ cell ml^{-1})	1.39	0.38	1.27
TOC (µM)	88.40	77.31	89.29
TOC:TIN:TIP	1768:46:1	1546:45:1	341:17:1
POC (µM)	4.87	8.51	7.73
TDCu (nM)	n.d.	n.d.	21.75

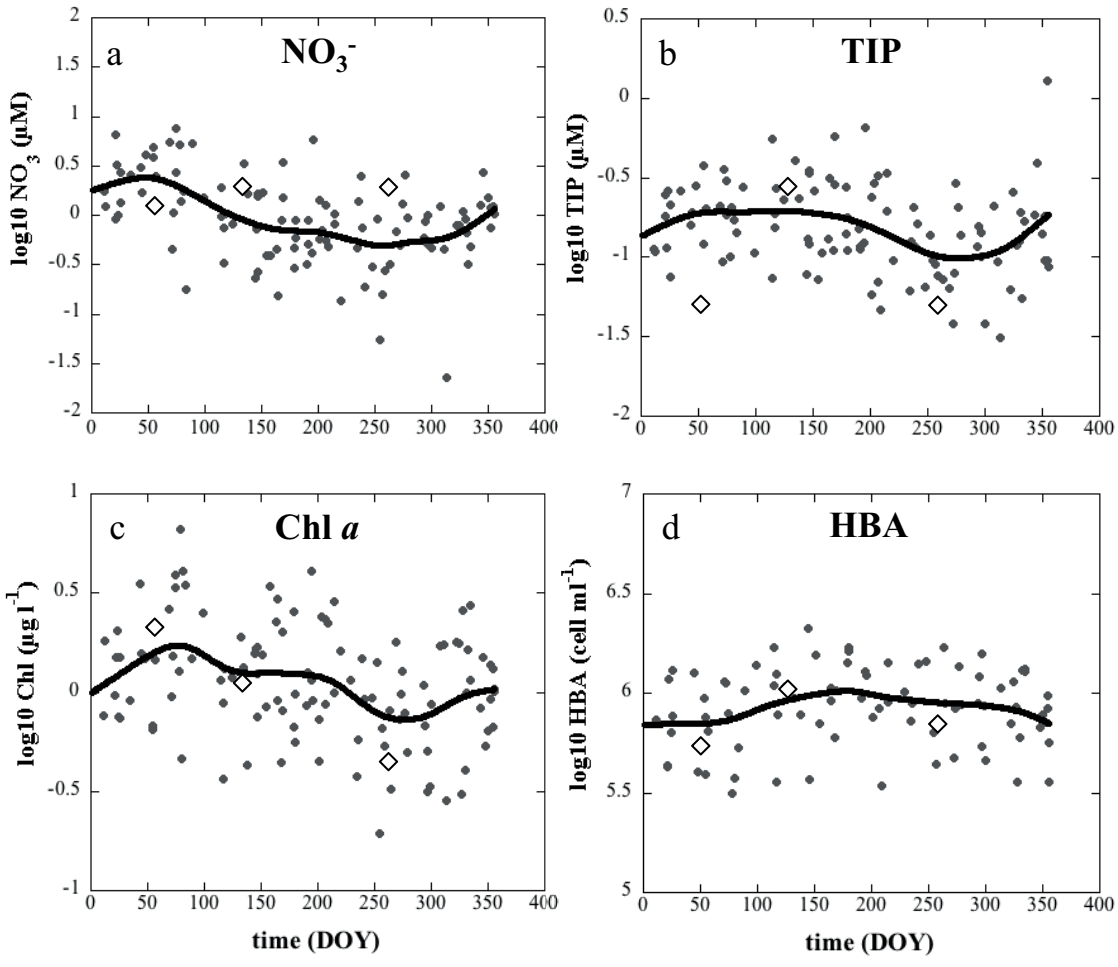

Figure 3.1. Concentration of nitrate (a), TIP (b), Chl *a* (c), and HBA (d) throughout the year in the surface waters of the Barcelona coastal area. The gray dots represent direct measurements at our sampling station from a 10-year time series, and the black line is a cubic spline fit. The white rhombi represent the concentration of a certain variable at the beginning of the winter (DOY=56), spring (DOY=132), and summer experiments (DOY=261).

3.3 Macronutrients dynamics

The evolution in the concentration of TIN and TIP in the different treatments throughout the three experiments is shown in Fig. 3.2. One hour after the aerosol addition, a remarkable increase in the concentration of TIN was usually observed in the amended microcosms, especially in the A ones, remaining almost constant afterwards in summer and winter but quickly decreasing in spring. On the contrary, smaller increases in TIP were observed after the amendment in the three experiments, and then it decreased toward the end of the experiment. The TOC concentration slightly increased after the amendment in all the experiments and then remained almost constant up to the last day (data not shown).

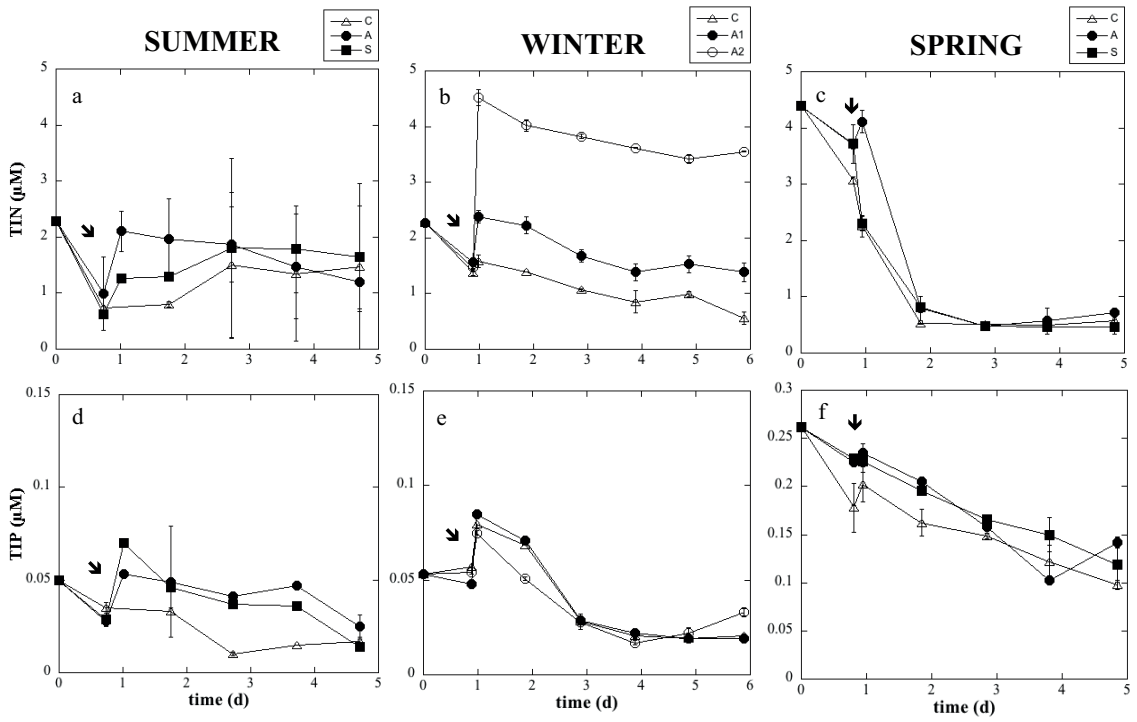

Figure 3.2. Concentration of inorganic nutrients throughout the incubation time in the three experiments: TIN in summer (a), winter (b), and spring (c); TIP in summer (d), winter (e), and spring (f). Error bars

represent the standard deviation between the two replicate containers. The arrow indicates the time of aerosol addition. Note the change in scale in f compared with d and e.

Table 3.2 shows the change in the nutrient concentration in Saharan and anthropogenic microcosms observed after adding the aerosols. Saharan dust produced an increase of 0.75 µmol in TIN and 0.05 µmol in TIP per mg of dust during summer, whereas a decrease in TOC was observed in this experiment after the dust addition. In contrast, in spring, an increase of 7.72 µmol in TOC per mg of Saharan dust was determined. No increase in P and a decrease in the nitrate and ammonium concentrations after the addition were detected during the spring experiment. These observations are further discussed in Section 3.4. On the other hand, an increase in the concentration of nitrate, ammonium, phosphate, and TOC was always observed in A microcosms after the amendment. On average, anthropogenic aerosols increased TIN by 1.67 ± 1.49 µmol mg^{-1}, TIP by 0.03 ± 0.02 µmol mg^{-1}, and TOC by 6.84 ± 1.33 µmol mg^{-1}. It must be noted that changes observed in nutrient concentrations in A and S microcosms (Table 3.2) were not always in the same proportion as they were in A and S filters (Table S3.3), respectively, presumably due to variations in the release of airborne nutrients from the filters. On average, 49 ± 37% of TIP, 39 ± 14% of TIN, and 29 ± 15% of TOC were recovered from the collected filters.

Table 3.2. Change produced in the concentration of nutrients in the amended microcosms (A and S) 1 h after the aerosol addition (expressed as µmol per mg of aerosol added). n.d.: not determined.

SEASON	AEROSOL	TOC	NO_3^-	NH_4^+	PO_4^{3-}	N:P
SUMMER	A	2.36	0.88	0.16	0.02	43
	S	−0.68	0.57	0.18	0.05	15
WINTER	A1	9.39	1.01	0.29	0.05	25

	A2	6.85	2.37	1.48	0.03	145
SPRING	A	8.80	0.33	0.17	0.01	41
	S	7.72	−1.48	−0.17	0.00	n.d.
AVERAGE	A	6.84 ± 1.33	1.15 ± 0.86	0.55 ± 0.61	0.03 ± 0.02	
	S	7.72 (SP)	0.57 (SU)	0.18 (SU)	0.05 (SU)	

The aerosol-induced ratios (AIRs) for each variable are presented in Table 3.3. AIR_{TIN} and AIR_{TIP} were quite above 1 in summer and winter. In spring, though, TIP was always below 1, and so it was TIN in the Saharan microcosms, pointing to a decrease in nutrient concentration after the aerosol addition during this experiment. AIR_{TIN} values were normally higher in A containers than in S, and the opposite was the case for AIR_{TIP}, but differences in AIR_{TIN}, AIR_{TIP}, and AIR_{TOC} between treatments were not significant (Table 3.4). Significant differences in AIR_{TIP} were found between seasons, with the highest AIR_{TIP} values determined in summer and the lowest in spring, in good agreement with the high P content determined in the filters collected in summer (Table S3.3).

Table 3.3. Mean aerosol-induced ratio (AIR) values ± standard deviation of the different biogeochemical variables measured in the three experiments. Values refer to a given aerosol-treatment (A, S) with respect to the controls.

	SUMMER		**WINTER**		**SPRING**	
	A	S	A1	A2	A	S
AIR_{TIN}	2.49 ± 1.28	1.96 ± 0.06	1.32 ± 0.09	2.67 ± 0.06	1.51 ± 0.07	0.84 ± 0.03
AIR_{TIP}	1.95 ± 0.07	2.48 ± 0.39	1.27 ± 0.01	0.99 ± 0.06	0.92 ± 0.05	0.87 ± 0.05
AIR_{TOC}	1.00 ± 0.09	0.97 ± 0.10	1.14 ± 0.00	1.12 ± 0.10	1.01 ± 0.03	1.00 ± 0.02
AIR_{Chl}	2.24 ± 0.23	2.15 ± 0.22	1.05 ± 0.00	1.06 ± 0.01	1.40 ± 0.06	1.17 ± 0.02
AIR_{Syn}	1.06 ± 0.00	1.10 ± 0.01	1.00 ± 0.01	0.98 ± 0.00	1.02 ± 0.05	0.97 ± 0.12

AIR_{Pico}	1.17 ± 0.02	1.10 ± 0.02	1.02 ± 0.00	1.01 ± 0.01	1.18 ± 0.18	1.07 ± 0.13
AIR_{Nano}	1.06 ± 0.03	1.08 ± 0.07	1.04 ± 0.01	1.08 ± 0.04	1.50 ± 0.03	1.04 ± 0.08
AIR_{HBA}	1.01 ± 0.01	1.03 ± 0.01	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.01	1.01 ± 0.00
AIR_{HBP}	1.84 ± 0.39	1.46 ± 0.22	1.24 ± 0.26	1.02 ± 0.02	1.40 ± 0.07	1.14 ± 0.00
AIR_{HNF}	0.98 ± 0.00	0.94 ± 0.01	1.12 ± 0.09	1.06 ± 0.24	1.00 ± 0.02	1.00 ± 0.01

Table 3.4. Results of the two-way ANOVA performed with AIR values (number of data, N =12). The factors were the kind of aerosol added (AE) and the season (SE). The R^2 adjusted and the p-value of the model are detailed for each of the variables. Tukey HSD test was performed when significant differences were found between seasons. SU= summer; WI= winter; SP= spring. *p<0.05; **p<0.01; ***p<0.001; n.s.: non-significant (p>0.05).

	ANOVA 1				Tukey HSD		
	R^2 adj	p-value	AE	SE	[SU-SP]	[SP-WI]	[SU-WI]
AIR_{TIN}	0.28	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
AIR_{TIP}	0.88	***	n.s.	***	SU>SP***	n.s.	SU>WI***
AIR_{TOC}	0.48	*	n.s.	n.s.	n.s.	n.s.	n.s.
AIR_{Chl}	0.95	***	n.s	***	SU>SP***	n.s.	SU>WI***
AIR_{Syn}	0.34	n.s.	n.s.	n.s	n.s.	n.s.	n.s.
AIR_{Pico}	0.33	n.s.	n.s.	n.s	n.s.	n.s.	n.s.
AIR_{Nano}	0.48	*	A>S*	n.s.	n.s.	n.s.	n.s.
AIR_{HBA}	0.65	**	S>A*	*	n.s.	n.s.	SU>WI*
AIR_{HBP}	0.59	*	n.s.	**	n.s.	n.s.	SU>WI*
AIR_{HNF}	0.33	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

3.3.4 Total dissolved copper (TDCu) dynamics

In the spring experiment, the concentration of TDCu in the seawater after the aerosol additions increased by 1.23 (± 0.27) times in the S containers with respect to C and by 1.61 (± 0.16) times in the A. In S microcosms, the concentration of Cu at the end of the

experiment remained similar to that immediately after the amendment, but it decreased from 47 to 34 nM in the A containers, indicating that 13 ± 1 nM of TDCu may either have been incorporated into the marine biota or settled down, attached to non-living particles.

3.3.5 Autotrophic community dynamics

An increase in chlorophyll *a* was observed in the amended microcosms in all the experiments, especially at the end of the experiment in summer and ca. 24 after the addition in spring (Fig. 3.3). The AIR_{Chl} was always above 1, indicating that both kinds of aerosols always yielded a positive effect on phytoplankton growth, although in winter they had almost no effect (Table 3.3). Overall, autotrophic picoplankton (*Synechococcus* and picoeukaryotes) behaved similar to both types of atmospheric particles (Fig. 3.4), whereas statistically significant differences were found in the response of nanoeukaryotes, which were more stimulated with anthropogenic aerosols than with Saharan dust (Table 3.4). While AIR_{Pico} and AIR_{Nano} were almost always above 1, AIR_{Syn} was close to 1 or even lower during winter and spring (Table 3.3). On the other hand, we identified *Prochlorococcus* at the beginning of all the experiments (Table 3.1), but 2 days after the aerosol addition, we were unable to detect them. As a similar decrease was observed in all the microcosms (aerosol-amended and controls), it is not possible to attribute cyanobacteria demise to aerosol toxicity. In any case, we will further discuss the potential toxicity of atmospherically derived copper on cyanobacteria later (Section 3.4.2).

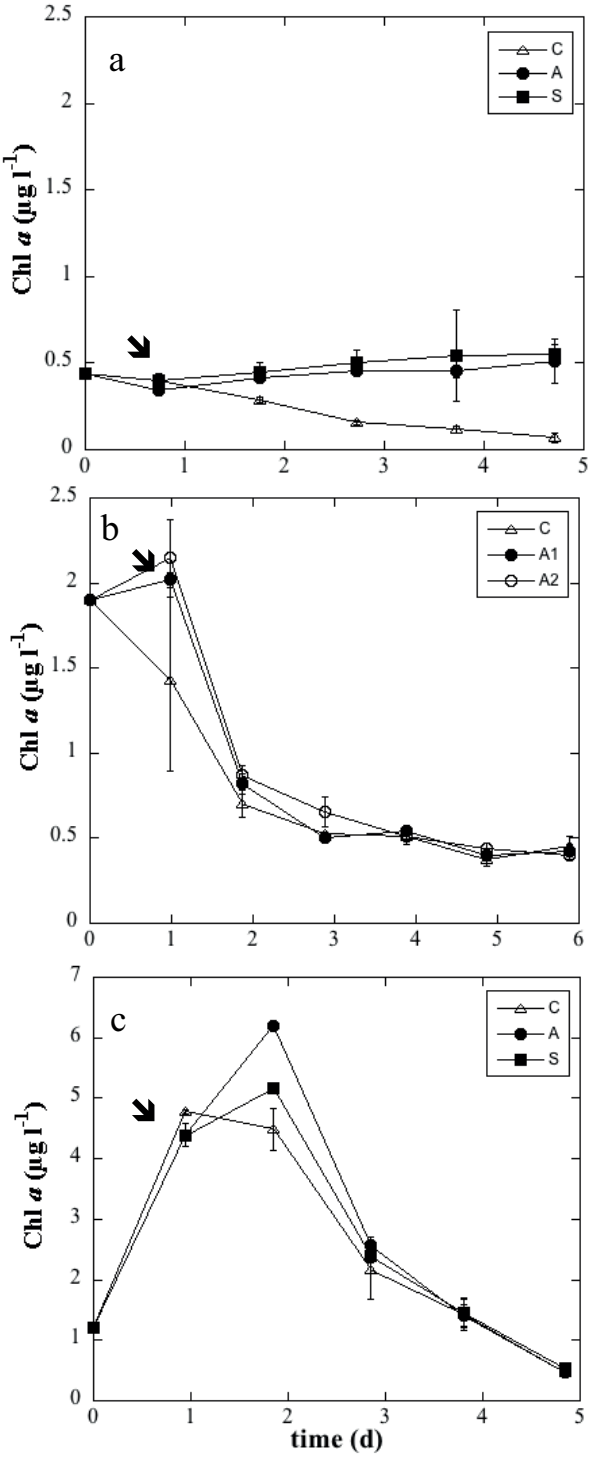

Figure 3.3. Concentration of Chl *a* throughout the incubation time in the three experiments: a) summer, b) winter, and c) spring. Error bars represent the standard deviation (n = 2). The arrow indicates the time of aerosol addition. Note the change in scale in c compared with a and b.

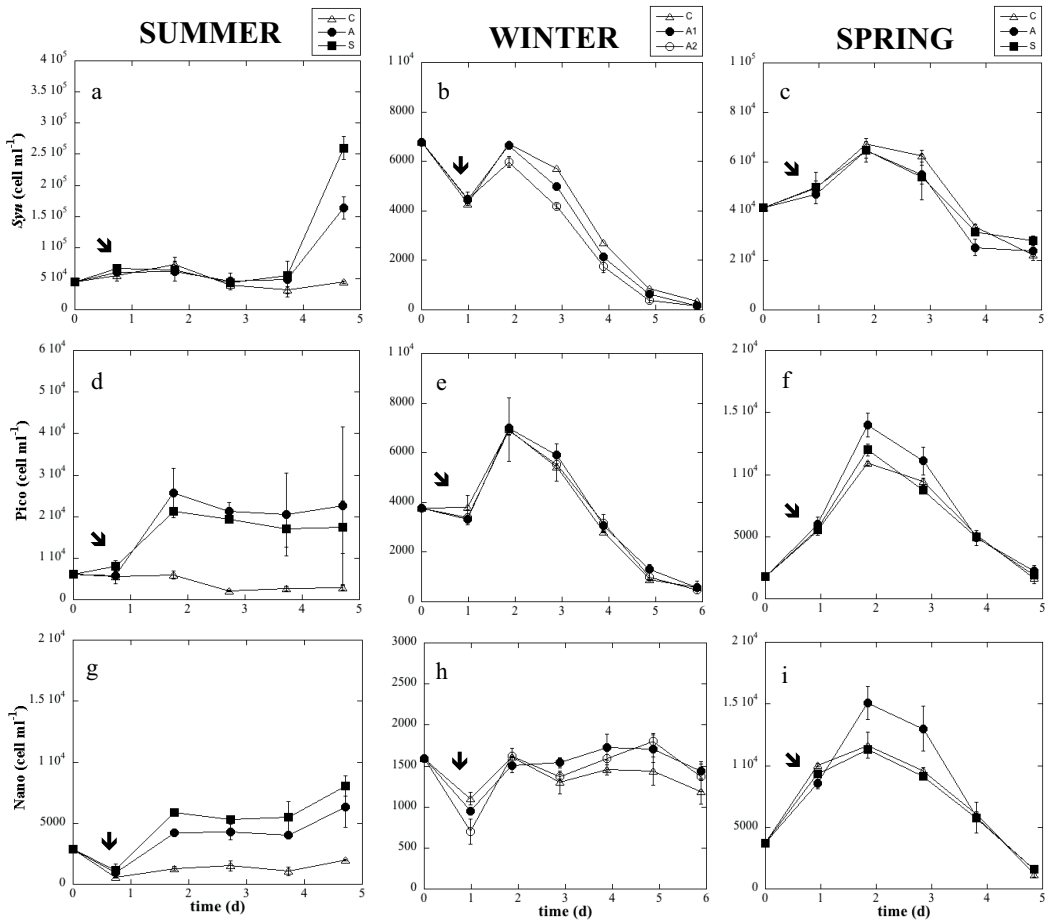

Figure 3.4. Concentration of *Synechococcus* (*Syn*), picoeukaryotes (Pico), and nanoeukaryotes (Nano) throughout the incubation time in the three experiments: *Syn* in summer (a), winter (b), and spring (c); Pico in summer (d), winter (e), and spring (f); Nano in summer (g), winter (h), and spring (i). Error bars represent the standard deviation (n = 2). The arrow indicates the time of aerosol addition. Note the changes in scale.

Significant differences in AIR_{Chl} were determined between seasons (Table 3.4). The highest increase was observed in summer and the lowest in winter. Although pico- and nanophytoplankton groups showed some differences in the amended microcosms with respect to the controls in summer, no statistically significant differences were determined between seasons. On the other hand, on comparing the relative effect of nutrient additions for the different types of aerosols, we found that most of the variables showed ratios below 1 when divided by both TIN and TIP and especially by TIN

(Supplementary material, Table S3.5). This is likely due to the high supply of N from both aerosols, especially from the anthropogenic ones. Nano/TIP (AIR_{Nano} divided by AIR_{TIP}) was still significantly higher in the A than in the S microcosms (Table 3.5), due to the similar (slightly higher in the S) concentration of P supplied by both types of atmospheric particles. Statistically significant AIR values were determined between seasons for all the phytoplankton groups when divided by TIP, the highest ratios always observed in spring. Nano/TIN was also significantly higher in spring than in summer and winter, given the low supply of both nutrients observed during spring and the higher AIR_{Nano} determined in spring (Table 3.3).

3.3.6 Heterotrophic community dynamics

Bacterial abundance showed an overall slight increase in the amended microcosms compared with the controls (Figs. 3.5a–c), AIR_{HBA} being always very close to 1 (Table 3.3). However, a significantly higher response was observed in the S than in the A containers (Table 3.4). In contrast, a marked peak in HBP was observed in the microcosms amended with anthropogenic particles during summer and spring and in A1 in winter, where the increase observed in P after the aerosol addition was higher (Figs. 3.5d–f, Table 3.3). The smallest fractions of HNF (<5; 5–10 μm) usually showed an increase toward the end of the experiments (Fig. 3.6). AIR_{HBA} and AIR_{HBP} were significantly higher in summer than in winter, whereas no significant differences were observed for AIR_{HNF} between seasons (Table 4). No statistically significant differences were found between both kinds of aerosols for either AIR_{HBP} or AIR_{HNF}.

Table 3.5. Results of the two-way ANOVA applied to the quotient derived from dividing AIR values of biological variables by AIR of nutrients ($N = 12$). The factors and data presented are the same as in ANOVA1 (Table 4). *p<0.05; **p<0.01; ***p<0.001; n.s.: non-significant (p>0.05).

	ANOVA 2				Tukey HSD		
	R^2 adj	*p*-value	AE	SE	[SU-SP]	[SP-WI]	[WI-SU]
Chl/TIN	0.53	*	n.s.	n.s.	n.s.	n.s.	n.s.
Chl/TIP	0.72	**	n.s.	**	SP>SU*	SP>WI*	n.s.
Syn/TIN	0.46	*	n.s.	n.s.	n.s.	n.s.	n.s.
Syn/TIP	0.85	***	n.s.	***	SP>SU***	SP>WI*	WI>SU**
Pico/TIN	0.49	*	n.s	*	n.s.	n.s.	n.s.
Pico/TIP	0.81	***	n.s.	***	SP>SU***	SP>WI*	WI>SU**
Nano/TIN	0.68	**	n.s.	**	SP>SU**	SP>WI**	n.s.
Nano/TIP	0.87	***	A>S*	***	SP>SU***	SP>WI*	WI >SU*
HBA/TIN	0.53	*	n.s	n.s	n.s.	n.s.	n.s.
HBA/TIP	0.88	***	n.s.	***	SP>SU***	SP>WI*	WI >SU***
HBA/TOC	0.47	*	n.s.	n.s.	n.s.	n.s.	n.s.
HBA/Chl	0.97	***	S>A*	n.s.	n.s.	n.s.	WI >SU***
HBP/TIN	0.33	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
HBP/TIP	0.82	***	A>S*	***	SP>SU**	SP>WI*	n.s.
HBP/TOC	0.55	*	n.s.	*	n.s.	n.s.	SU>WI *
HBP/Chl	0.49	*	n.s.	*	n.s.	n.s.	WI>SU*
HNF/TIN	0.44	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
HNF/TIP	0.89	***	n.s.	*	SP>SU***	n.s.	n.s.

As observed with the autotrophic community, significantly larger increases in AIR_{HBA}, AIR_{HBP}, and AIR_{HNF} were estimated in spring than in the other seasons when divided by AIR_{TIP} (i.e. HBA/TIP, HBP/TIP and HNF/TIP), and a greater HBP/TIP was found in A than in S microcosms, as a consequence of the higher AIR_{HBP} observed in A microcosms and the opposite pattern observed for AIR_{TIP} (Table 3.3 and 3.5). AIR_{HBA}

was still significantly higher in the S than in the A microcosms when divided by AIR_{Chl} (HBA/Chl), given the higher increase in HBA observed in the S and the opposite pattern observed for Chl *a*. HBA/Chl and HBP/Chl were significantly larger in winter than in summer, whereas the opposite was observed for HBP when divided by TOC.

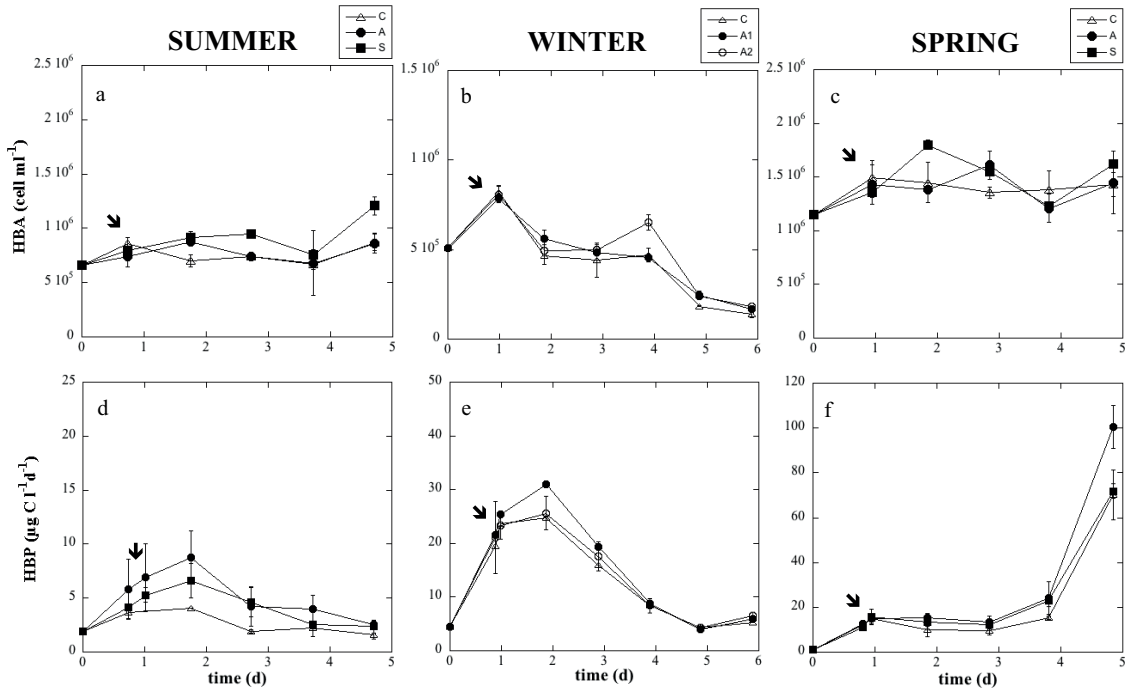

Figure 3.5. HBA and HBP throughout the incubation time in the three experiments: HBA in summer (a), winter (b), and spring (c); HBP in summer (d), winter (e), and spring (f). Error bars represent the standard deviation (n = 2). The arrow indicates the time of aerosol addition. Note the changes in scale.

3.3.7 Distribution of POC among biological compartments

In all seasons, the highest percentage of POC measured at the beginning of the experiment was that of phytoplankton. The autotrophic community was clearly predominant in winter, followed by spring, and then summer. The sum of autotrophic and heterotrophic components was always less than 100%, the remaining carbon belonging to non-determined marine microorganisms (e.g., microzooplankton other than HNF), to detrital POC present in the seawater, and to non-living organic particles

supplied by the aerosols themselves (in the case of the amended microcosms). Figure 3.7 shows the distribution of POC at the beginning (T_0) of each experiment and in each treatment at the end. Values have been normalized at 100% for each microcosm.

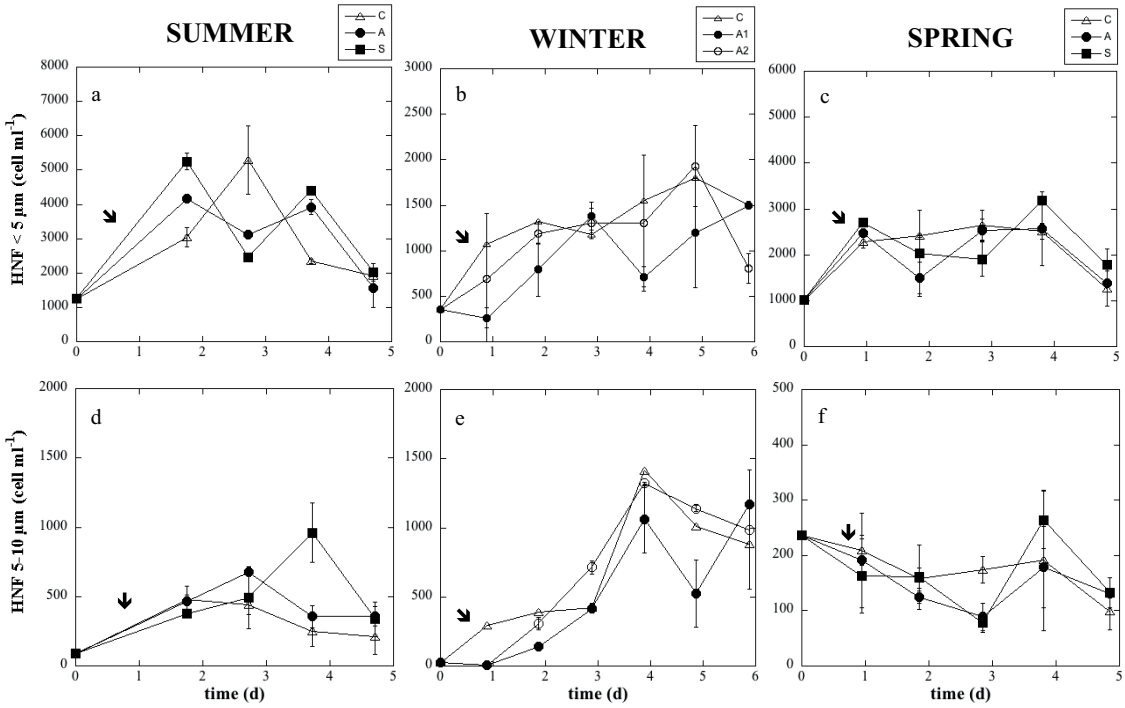

Figure 3.6. HNF throughout the incubation time in the three experiments: HNF <5 µm in summer (a), winter (b), and spring (c); HNF sized between 5 and 10 µm in summer (d), winter (e), and spring (f). Error bars represent the standard deviation (n = 2). The arrow indicates the time of aerosol addition. Note the changes in scale.

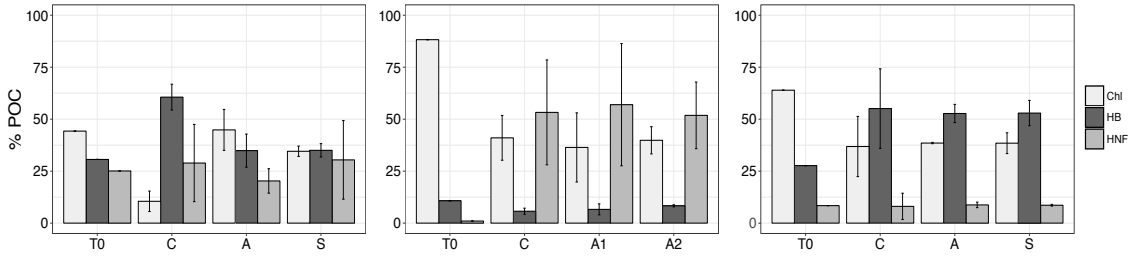

Figure 3.7. Biomass distribution of phytoplankton (as derived from Chl *a*), heterotrophic bacteria (HB), and heterotrophic nanoflagellates (HNFs), expressed as a percentage of POC, at the beginning (T_0), and at the end of each experiment in the different microcosms. Data are normalized to 100%. Error bars represent the standard deviation (n = 2).

In the summer experiment, phytoplankton biomass accounted for 44% and HB for 31% of the initial biological POC. HNF biomass was remarkably high and constituted 25% of the initial POC. At the end of the experiment, the contribution of phytoplankton was reduced in all the microcosms, but it decreased much more in the controls, where HB became the major biological component of the particulate carbon, followed by HNF. Phytoplankton biomass was the main contributor of POC in the A microcosms, while in the S, the percentage of all the three components was similar.

Autotrophic biomass constituted 88% of the POC at the beginning of the winter experiment, whereas bacterial biomass and HNF contributed only 11% and 1%, respectively. At the end of the experiment, the distribution changed completely in all the microcosms. Phytoplankton was reduced to 36% and 40% in A1 and A2, respectively, and to 41% in C, while HNF became the main contributors to biological POC, especially in A1. The percentage of bacterial biomass was slightly higher in the anthropogenic microcosms than in the controls.

The situation during spring was intermediate between the two other experiments. Phytoplankton biomass accounted for 64% of the initial POC, HB biomass contributed 28%, and HNF 9%. A sharp decrease in phytoplankton biomass was observed at the end of the experiment, similarly in all the containers, while HB became the main contributors and HNF remained in a similar proportion to initial conditions.

3.4 Discussion

3.4.1 Implication of aerosol composition on nutrient dynamics

Overall, the collected filters showed a chemical composition within the ranges

previously reported in Barcelona (Pey et al., 2010; Querol et al., 2001; Viana et al., 2005). The concentrations of the elements and compounds determined in Saharan dust were always higher than in anthropogenic aerosols as was the concentration of TSP collected in these filters, but, proportionally, anthropogenic aerosols were richer in N and especially in OC (Table S3.3). Both anthropogenic and Saharan aerosols presented a N:P molar ratio quite above Redfield's, although the values were lower than those reported by Markaki et al. (2010) in the western Mediterranean, a finding that may be attributed to the proximity of our sampling point to local anthropogenic sources. It must be pointed out that the collected aerosols always showed a mix of components including local sources and that it was not possible to collect pure Saharan dust in a large urban location such as Barcelona. Nonetheless, the observed compositions were representative of depositions over the Barcelona coast and thus adequate to study their effects in the urban coastal environment.

In our experiments, we found that in summer, the high P content supplied by both types of aerosols contributed to alleviating the extreme P limitation occurring at the beginning of the experiment. Contrarily, the great amount of N supplied to the water by the anthropogenic particles in winter produced a large increase in TIN, which could not be assimilated by the biota because it soon became P-limited (Fig. 3.2b–3.2e). TIN:TIP increased through the experiment up to 75 and 108 in A1 and A2, respectively. These results are in good agreement with other nutrient-addition experiments carried out in the Mediterranean Sea, where P was observed to be the main limiting nutrient (Guadayol et al., 2009; Pinhassi et al., 2006; Zweifel et al., 1993). In spring, the high initial concentration of both TIN and TIP determined at the start of the experiment allowed both phytoplankton and HB to grow in all the microcosms before the amendments (Fig.

3c, 4c, f–i, and 5c). Then, when adding the aerosols, the relatively low amounts of N and P supplied by the Saharan dust and the high turnover rate of P (Herut et al., 2005; Ridame and Guieu, 2002; Romero et al., 2011) enabled us to detect an increase in these nutrients after the addition. The increase in both nutrients in the anthropogenic microcosms was also lower than that in the other experiments. Excluding the spring data, the average increases in N and P produced with the aerosol additions (Table 3.2) are quite above those previously reported in the NW Mediterranean for N and P from Saharan dust (Herut et al., 2005; Ridame and Guieu, 2002; Romero et al., 2011). However, Duarte et al. (2006) observed a much greater increase in both N (24.69 μmol mg^{-1}) and P (1.73 μmol mg^{-1}) from dry dust, similar to the increase in N supplied by anthropogenic aerosols reported by Bonnet et al. (2005) (24 μmol mg^{-1}). On the other hand, Guo et al. (2014) estimated an increase of 1 μmol mg^{-1} for N released from anthropogenic particles, similar to the average value determined in our experiments (1.67 μmol mg^{-1}).

These differences observed in the nutrient supply are due to two reasons. First, the chemical composition of both anthropogenic and Saharan filters may be highly variable (Law et al., 2013; Moreno et al., 2006). We observed high differences in the chemical composition of the collected filters (Table S3.3), even if they were obtained in 2 consecutive days. This heterogeneity explains why, when considering the ratios between the AIR of biological parameters to the AIR of the nutrients (mainly AIR_{TIP}), we found a significantly higher increase in spring than in winter and summer (in spite of the larger AIRs determined in summer for most of the biological variables). On the other hand, different methodologies used for aerosol collection and particle extraction might also yield different results. Wet deposition collection using nets has been

employed in previous experiments (e.g., Marañón et al., 2010), but it has been observed that these nets preferentially select larger aerosol particles (Glaccum and Prospero, 1980) and that the rainwater of the wet deposition may wash out part of the soluble nutrients present in the aerosols (Romero et al., 2011). Another strategy, used, for example, during DUNE experiments (see Guieu et al., 2010b), was the artificial production of dust from soils collected in the Saharan region to keep the fine fraction. This method has the advantage of working with fixed proportions of N and P, but does not represent real variability in the concentration of nutrients observed in the atmosphere. Duarte et al. (2006) performed amendment experiments, adding dry particles using a methodology similar to ours. The main problem associated with this methodology is that the release of nutrients to the water may be in a different proportion than in the atmospheric filters, and it is difficult to determine if these differences should be attributed to variations in the solubility of the different nutrients or to methodological issues. We found some variations in nutrient release (29–49%, see Section 3.3) from both kinds of aerosols, but overall the TIN and TIP supplied by both types of aerosols were representative of what had been analyzed in the filters, TIN in higher proportion in the anthropogenic microcosms, whereas a similar TIP concentration was measured in both amended microcosms after the additions (Table 3.2 and S3.3). Nonetheless, as less than 50% of all of them were recovered, our results would be in any case underestimating the response of microorganisms to both anthropogenic and Saharan particles. Another option would be to directly add the sampled filters into the experimental recipients as done by Ternon et al. (2011). This probably assures a higher percentage of nutrient release but presents the disadvantage that particles can take up 24 h to be released while the experiment is being carried out, making it difficult to distinguish the fraction of dissolved nutrients attributed to the aerosols versus that due

to re-mineralization.

The initial concentration of TDCu determined in the seawater (21.75 nM) was one order of magnitude higher than that reported for the global ocean (2.5 nM; Rauch and Pacyna, 2009) and in the Strait of Gibraltar (3.6 nM; Boyle et al., 1985), but it was within the range values reported by Rossi and Jamet (2008) in the coastal waters of southeastern France (3.62–44.37 nM). We also measured a similar concentration of TDCu in another, less populated, coastal location of NE Spain. High copper concentrations in Mediterranean coastal waters are a consequence of the high inputs received from rivers and atmospheric deposition, plus local sources (Durrieu de Madron et al., 2011; Martin et al., 1989). Bruland et al. (1991) determined that dissolved copper was necessary for marine microorganisms in a P:Cu relationship of 1:0.0004. We determined a Cu/P ratio of 0.08 at the beginning of the spring experiment, pointing that the coastal waters near the shore of Barcelona are Cu-enriched for marine planktonic microorganisms. Furthermore, Jordi et al. (2012) established a threshold for phytoplankton growth inhibition at a Cu concentration above $8.23.10^{-5}$ nM in the NW Mediterranean, a concentration much lower than that reported in this study, pointing to a possible toxicity of phytoplankton in Mediterranean coastal waters. It is noteworthy that the effect is highly species dependent, though (Durrieu de Madron et al., 2011; Jordi et al., 2012). Nonetheless, as all the treatments started with the same seawater composition, and we aim to compare differences relative to controls, in the next section, we will consider the effect of TDCu released by the aerosols on marine cyanobacteria.

3.4.2 Effect of aerosols on the autotrophic community

The larger increase observed in phytoplankton occurred in summer. This is probably related to both the low Chl *a* concentration determined at the beginning of this experiment, even lower than that predicted at that time of the year (Fig. 3.1c), and to the P supply by the aerosols, as explained in Section 3.4.1. During winter, the high concentration of Chl *a* and the low concentration of P detected at the beginning of this experiment (Fig. 3.1 and Table 3.1), combined with the high N:P ratios determined in the aerosols collected in winter (Table S3.3), implied that the amendment was not sufficient to substantially stimulate the growth of phytoplankton, only observing a slight increase in the A microcosms compared with the controls (Fig. 3.3b). In spring, both TIN and TIP present in the seawater had been consumed before the aerosol addition. Then, after the amendment, the N:P ratio decreased in all the microcosms below Redfield's except in the A, the decrease being sharper in the controls than in the S microcosms. Consequently, a noticeable peak in Chl *a* was observed 1 day after the amendment in the enriched microcosms, particularly in the A, at the same time as TIN decreased (Fig. 3.2c and 3.3c). However, when comparing the observed increases in the phytoplankton at the same TIP addition, significantly larger ratios were observed in spring for all the phytoplankton fractions (Table 3.5). These results evidence that, although atmospheric deposition is likely to produce a major stimulation in plankton dynamics during the stratification period (i.e., in summer) because of the lower basal concentrations of nutrients and the importance of atmospheric deposition events versus other sources at this time of the year, atmospheric inputs also have the potential to trigger phytoplankton growth at other times of the year.

On the other hand, a potential toxic effect of aerosols on *Prochlorococcus* and

Synechoccocus has recently been suggested, attributed to their high content in trace metals (Paytan et al., 2009; Marañón et al., 2010). In the eastern Mediterranean, Herut et al. (2005) encountered a negative correlation between Saharan dust and *Prochlorococcus*, although they found a positive effect on *Synechococcus*. Mann et al. (2002) observed a toxic effect of Cu on natural populations of *Prochlorococcus* while Paytan et al. (2009) observed the same on *Synechococcus*. The latter determined a toxicity threshold between 0.2 and 2 µg of Cu per µg of Chl *a* on *Synechococcus* WH8102. If we considered that the complete decrease in the copper concentration (13 nM) determined at the end of the spring experiment was incorporated into *Synechococcus* cells and that their biomass after the amendment was ca. 8 µg l^{-1} in A containers, considering a conversion factor of 50 µg C per µg of Chl (Section 3.2.4), a concentration of 5 µg of Cu would be incorporated per µg of Chl *a* on *Synechococcus,* a value quite above the toxic limit established by Paytan et al. (2009). However, the decrease in *Synechococcus* biomass determined since the aerosol addition in the A containers was lower than in the S and the controls. Hence, there is no proof of atmospheric copper toxicity on *Synechococcus*, and TDCu from aerosols may have settled down attached to non-living particles. Furthermore, both *Synechococcus* and *Prochlorococcus* showed a decreasing pattern in the amended microcosms similar to that observed in the controls either in winter or spring, the AIR value very close to 1. Thus, the observed results point to other possible causes of the cyanobacteria demise as suboptimal light conditions inside the microcosms, viral attacks (Pulido-Villena et al., 2014), or high grazing pressure by HNFs (Guo et al., 2014). The inability to detect *Prochlorococcus* after 2–3 days of incubation may simply be due to their sensitivity to handling (Partensky et al., 1999). In any case, it must be noted that we only had TDCu data from the spring samples, which was not enough to do an evaluation of significance.

Thus, considering the threshold toxic values of copper determined by Jordi et al. (2012) in the NW Mediterranean, the effect of dissolved Cu supplied by atmospheric particles on Mediterranean phytoplankton (and especially cyanobacteria, due to their higher sensitivity) needs to be more thoroughly evaluated. The interactions between the different metals supplied by aerosols should be considered when evaluating the toxicity levels of Cu, as antagonistic effects between copper and other metals have been documented (Mann et al., 2002, and references therein).

Overall, our results showed that both kinds of aerosols produced an increase in the phytoplankton concentration compared with the controls and that the effect on nanoeukaryotes was significantly larger with anthropogenic particles. The stimulation was still significantly larger in the A microcosms when divided by TIP, as we found a similar supply of TIP from both sources. This is in good agreement with the results reported by Bonnet et al. (2005) and Ternon et al. (2011) in Mediterranean waters, who observed a larger increase in the small phytoplankton size-fractions with anthropogenic particles than with Saharan dust. Therefore, if any potentially toxic effect of trace metals supplied by aerosols (mainly from anthropogenic sources) exist, our results suggest that it is outweighed by the aerosol content in N and P, which are the main limiting nutrients in Mediterranean surface waters. Béthoux et al. (2002) predicted a change in the phytoplankton composition in Mediterranean waters because of the increase in N and P concentrations with respect to silicate from atmospheric and terrestrial inputs, triggering the growth of non-siliceous species of potential toxicity. In our experiments, we found a higher recovery of N and P from aerosols with respect to silicate, which favored the growth of non-siliceous species with respect to diatoms (Sdena Nunes personal communication). Thus, the current trend of increasing

atmospheric emissions from both anthropogenic and natural sources could withstand this pattern of composition change of phytoplankton species to a community dominated by smaller cells, with the risk of production of harmful algal blooms.

3.4.3 Effect of aerosols on the heterotrophic community

We found a significantly greater response of both HBA and HBP in summer than in winter. HBA response to Saharan dust was significantly larger than to anthropogenic particles. This suggests that not all the high amounts of OC supplied by the latter are useful for bacterial growth, although it must be considered that the percentage of TOC released from the anthropogenic filters was only 28 ± 18%. In the Saharan filters, 30% of TOC was released from the spring filter, whereas no increase in TOC was observed in summer after the aerosol addition. This is likely due to a combination of the lower concentration of TOC determined in the Saharan particles in summer (Table S3.3) and the overall lower solubility of Saharan dust with respect to anthropogenic aerosols. Apart from the low release of TOC, the analysis of dissolved organic matter in these experiments pointed out the recalcitrant character of the organic matter associated with the aerosols, as they contained a high proportion of chromophoric compounds that were not utilized along the incubation period (Sánchez-Pérez et al., 2016). As marine bacteria are known to have a lower N:P ratio than phytoplankton (Fagerbakke et al., 1996; Zweifel et al., 1993), their preference for Saharan dust versus atmospheric particles could alternatively be attributed to the higher amount of P supplied by the dust in summer (Table 3.2). This hypothesis is supported by the fact that TOC was in excess with respect to inorganic N and P at the beginning of all the experiments considering the theoretical ratio of 45:10:1 estimated for marine bacteria (Zweifel et al., 1993) (Table 3.1). In any case, these results may not be taken as conclusive, given the low AIR ratios

determined for HBA (Table 3.4), compared with the error of the analytical measurements (CV= 2%).

In previous aerosol-enrichment experiments, no effect or even HBA decreases were observed, while HBP was significantly stimulated (Duarte et al., 2006; Herut et al., 2005; Laghdass et al., 2011; Marañón et al., 2010). We also observed higher AIR_{HBP} ratios than AIR_{HBA} in all the seasons (Table 3.3). The major stimulation of bacterial production compared with that of bacterial abundance has been attributed either to a higher sensitivity of metabolic processes to external changes (Gasol and Duarte 2000) or to the effect of grazing on bacteria (e.g., Bonnet et al., 2005; Herut et al., 2005; Marañón et al., 2010). In the summer experiment, both reasons seem compatible. The higher concentration of the smaller fraction of HNF detected at the beginning of this experiment may have thwarted picoplankton growth until the end of the experiment, whereas HBP quickly responded to aerosols (especially to the anthropogenic; Fig. 3.5d). In winter and spring instead, maximal values of HNF followed the peak in autotrophic and heterotrophic picoplankton within a lag of 2–3 days (Fig. 3.4–3.6), similar to that reported by Guo et al. (2014). This points more to a bottom-up effect, as discussed in the next section.

On the other hand, a significantly larger stimulation of both HBA and HBP (as measured by AIR_{HBA} and AIR_{HBP}) was determined in spring than in summer and winter when divided by AIR_{TIP}, as observed with phytoplankton. Then it can be said that the biological response of the planktonic community of coastal waters is dependent on the initial conditions of the seawater as well as on the chemical composition of the aerosols and the solubility of the released particles in the seawater.

3.4.4 Response time and overall effect on the planktonic community

Figure 3.8 represents AIR values of chlorophyll *a* and bacterial abundance and production with respect to the time of maximum response since the aerosol addition in each independent microcosm (the time at which ABR_x was calculated, Eq. 3.2). Goldman (1993) showed that diatoms tend to be competitively superior in nutrient-replete environments because they are more capable of responding quickly to nutrient injections. In good agreement with this idea, the response of phytoplankton, mainly composed of diatoms (Sdena Nunes personal communication), to aerosols was the fastest in winter, although it was also the lowest in intensity (Fig. 3.8). On the contrary, the largest but slowest response of phytoplankton was observed in summer, when the smaller fractions were the dominant (i.e., cyanobacteria and picoplankton). These observations are in good agreement with the idea that smaller cells are usually better competitors in oligotrophic conditions due to their larger surface-to-volume ratio (Raven, 1998). Giovagnetti et al. (2013) also found a dominance of picophytoplankton in a summer experiment within the DUNE project, this fraction showing a quick response after a first dust seeding, while nanoeukaryotes responded more positively after a second seeding. This result would be comparable to what occurred during the spring experiment, where the phytoplankton biomass increased in all the microcosms before the addition of the aerosols, presumably due to the availability of both N and P already present in the seawater, and then, after the new pull of N and P supplied by the anthropogenic aerosols, nanoeukaryotes significantly increased their concentration in the A microcosms compared with the C and the S. Overall, our results agree with the others reported in the NW Mediterranean Sea in which a response of phytoplankton to dust was observed in spring (Romero et al., 2011) and summer (Laghdass et al., 2011)

but was very weak in winter (Bonilla-Findji et al., 2010). Laghdass et al. (2011) detected an average Chl *a* increase in Saharan dust-amended mesocosms of 1.83 with respect to the controls during the DUNE experiment, at the same added dust concentration as in our study. This is very similar to the averaged AIR values that we determined in summer, 2.15 and 2.24 in Saharan and anthropogenic aerosols, respectively (Table 3.3).

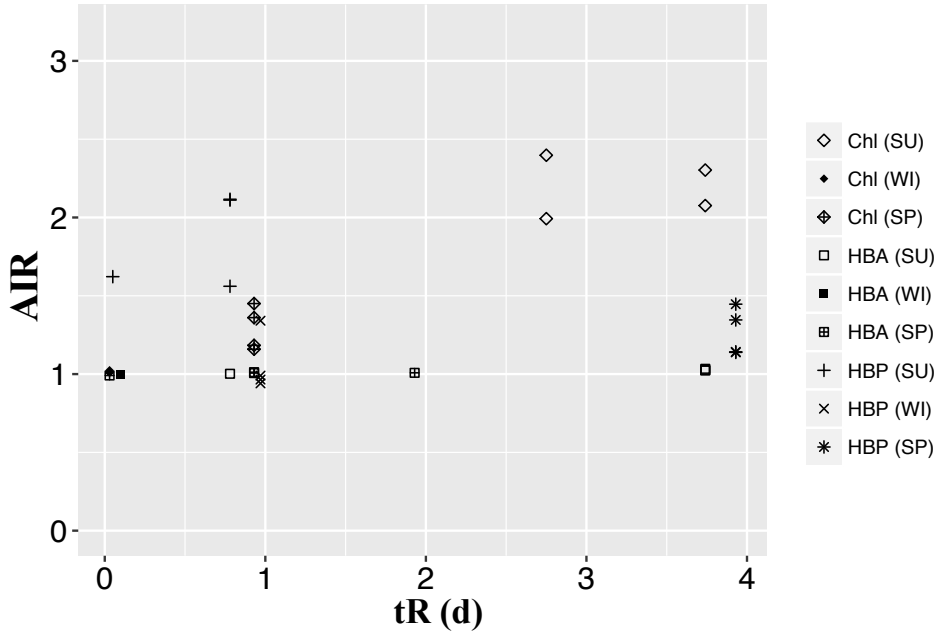

Figure 3.8. AIR values determined for Chl *a*, HBA, and HBP in all the experiments with respect to the time of response – tR (days) – since aerosol addition.

On the other hand, Marañón et al. (2010) observed that the response of bacterial production was faster and higher in intensity in the most oligotrophic locations, in good agreement with Raven's (1998) hypothesis. Accordingly, we observed that HBP became larger from winter to summer, showing a quicker response in summer. In spring and winter, though, maximal rates were always observed after the peak in Chl *a* (Fig. 3.8). A significantly higher increase in both HBA and HBP was observed in winter than in summer when divided by the increase in Chl *a*, whereas the opposite was observed for

HBP when divided by TOC (Table 3.5). These observations point out that, when available, bacteria take up organic carbon released from autotrophic microorganisms rather than from aerosols. Thus, results from these experiments suggest that bacterial metabolism is preferentially stimulated with aerosols (especially of anthropogenic origin) when there is a low concentration of labile organic matter in the seawater (i.e., in summer). On the contrary, in winter, given the high concentration of chlorophyll initially present in the seawater and also the high HBP, no stimulation of the heterotrophic production was observed with the anthropogenic aerosols, but it slightly increased when the chlorophyll started to decrease. The case of spring showed a similar result, although a higher stimulation than that in winter was observed as occurred with chlorophyll. These results are in good agreement with those reported by Pulido-Villena et al. (2014). These authors observed an increase in HBP concomitant with a decrease in P after 2 days of a first dust seeding in mesocosm experiments carried out in Corsica during summer. However, the extent in the increase of bacterial respiration during a second seeding, when P was not anymore being consumed, suggested an indirect effect after the increase in phytoplankton.

Overall, atmospheric particles produced a slight change in the net trophic balance that depended on their composition and the initial biogeochemical conditions in the experimental water. In summer, aerosols, especially of anthropogenic origin, turned a community that tended to heterotrophy into another one less heterotrophic, whereas the opposite was observed in winter (Fig. 3.7). However, the high standard deviations observed in winter for Chl and HNF prevented us from establishing a clear pattern. Duarte et al. (2004) and Martínez-García et al. (2015) also observed a larger stimulation of primary production versus respiration during the period of low nutrient availability in

a coastal location close to Barcelona and in the more productive NW coast of Spain, respectively. In contrast, during DUNE mesocosm experiments, Guieu et al. (2014) observed that the tendency to heterotrophy in the oligotrophic waters of Corsica remained or was even stimulated with Saharan dust. Considering that the former studies were performed in coastal places, the hypothesis of Marañón et al. (2010) about the degree of oligotrophy seems to adequately explain the (pico)phytoplanktonic community taking advantage of less oligotrophic waters than bacteria. On the other hand, Lekunberri et al. (2010) and Romero et al. (2011) found a shift toward heterotrophy in Barcelona coastal waters with the lowest concentration of dust added (50 mg l^{-1}) and toward autotrophy with the highest (500 mg l^{-1}). Thus, the question remains open, the final balance probably dependent on the initial characteristics of the seawater (basal concentration of nutrients, Chl, and HBA) and nutrients delivered by aerosols. More studies carried out at different times of the year are needed to evaluate the effect of aerosols on coastal locations.

Finally, it must be considered that the response of the biological variables in winter was only evaluated with respect to anthropogenic aerosols, when the effect of atmospheric particles was overall the lowest. This implies that anthropogenic inputs may have yielded even a larger stimulation than that of Saharan dust at the same deposition fluxes. Although, as mentioned earlier, concentrations of anthropogenically derived atmospheric particles of 0.8 mg l^{-1} are not currently realistic; deposition events of this magnitude could be reached in a short-term future. Thus, given their potential to stimulate both the autotrophic and heterotrophic communities at least equally as Saharan dust, more studies are needed to assess the effect of this type of particles on marine planktonic communities. This is of main interest in coastal areas, where the

influence of anthropogenic sources is greater, and so they are the services provided to their inhabitants (e.g., fishing, tourism).

3.5 Conclusions

Our data showed an overall increasing response to aerosols of both the autotrophic and heterotrophic communities from winter to summer. These responses are dependent on the biogeochemical status of the seawater, the chemical composition of the atmospheric particles, and their solubility in seawater. When the initial community was mainly heterotrophic, namely during summer, aerosols showed a tendency to turn the community toward autotrophy, whereas the opposite was observed during winter. Anthropogenic particles stimulated the growth of nanoeukaryotes significantly more than Saharan dust. The largest difference between aerosol treatments was observed in spring, related to the higher N content in anthropogenic aerosols and the P/N co-limiting conditions at the moment of aerosol addition, while the heterotrophic bacterial community seemed to be more dependent on the P content. The largest stimulation of bacteria occurred in summer, mainly due to the higher P supplied by the Saharan dust and their higher capacity of competition during more oligotrophic conditions, whereas in winter and spring they showed an indirect stimulation following the phytoplankton maximal growth.

In the present world, with a general trend of increasing anthropogenic emissions, especially from the emerging industrialized countries, and the risk of desertification due to the current climate change, it is important to ascertain the effects of atmospheric particles of different origins on the marine planktonic community as a whole. More experimental studies combined with synoptic observations are desirable to understand

the possible short-term and long-term implications of anthropogenic atmospheric inputs and their interaction with dust particles on the Mediterranean microbial communities. This is of special interest in the more anthropogenically influenced coastal areas, where there are many different sources of nutrients affecting the seasonal structure of the marine community.

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Supplementary material

Supplementary Methods. Detailed description of aerosol chemical determination and classification

Supplementary Table S3.1. Summary of the experimental design

Supplementary Table S3.2. Results of the ANCOVA performed for each measured variable in both C1 and C2 controls

Supplementary Table S3.3. Chemical composition of the collected filters

Supplementary Table S3.4. Ratios calculated for major elements and metals in the collected filters

Supplementary Table S3.5. Quotients of AIR of the biological variables divided by AIR of nutrient

Supplementary Methods. Detailed description of aerosol chemical determination and classification

Once the gravimetric determination of the particulate matter trapped in the filters was performed, they were cut into two equal sections. Half of the filter was kept at 4 ºC and employed to characterize the chemical composition of the particles and the other half was frozen at −20 ºC until it was used for the amendment experiments.

To do the chemical characterization, the first half of the filter was divided into two equal portions. One of them was acid-digested and analyzed for major and trace elements by inductively coupled plasma mass and atomic emission spectrometry, as described in Querol et al. (2001). The other quarter was further cut into two parts. One quarter of it was leached in 20 ml of milli-Q water for the extraction of water-soluble anions and subsequent analysis by ion chromatography for sulfate, nitrate, and chloride and by a specific electrode for ammonium. Finally, a section of 1.5 cm^2 from the other part was used for the determination of organic carbon by a thermal-optical transmission technique using a Sunset Laboratory OCEC Analyzer, following the procedure described in Moreno et al. (2006). Silica concentration was calculated from that of the aluminum, based on empirical factors ($3\times Al_2O_3=SiO_2$).

Once the chemical analyses were done, filters were classified as Saharan or anthropogenic based on element ratios following the criteria determined by Guieu and Thomas (1996), Guieu et al. (2002), Nava et al. (2012), and references therein. These authors defined the threshold mineral ratios of 0.012 for P:Al, 0.63 for Fe:Al, 1.01×10^{-3}

for Zn:Al, 3.41×10^{-4} for Pb:Al, and 1.71×10^{-6} for Cd:Al. Values above these ratios would correspond to anthropogenic origin aerosols. According to Nava et al. (2012), higher Si:Ca, Si:Fe, Al:Ca, Al:Fe, Ti:Ca, and Ti:Fe ratios are expected in the Saharan dust. In addition, the enrichment factor (EF) was calculated for trace metals as described in Migon et al. (2001):

$$EF_M = \frac{(\frac{M}{Al})_{AE}}{(\frac{M}{Al})_{CR}} \qquad \text{Eq.S3.1}$$

where *M* is the concentration of the metal in the aerosol (AE) or in the continental crust (CR). The concentrations of the elements in the CR are the ones reported by Wedepohl (1995). Ratios close to 1 are typical of crustal origin aerosols, while values above 10 normally belong to anthropogenically influenced aerosols (Migon et al., 2001).

Table S3.1. Experimental microcosms (C1, C2, A, or S as a function of the treatment received) and variables measured during the three experiments. Variables measured in each particular microcosm are signaled by an *X*, otherwise with a hyphen (-). Abbreviations: TOC = total organic carbon, Chl *a* = chlorophyll *a*, HBA = heterotrophic bacterial abundance, HBP = heterotrophic bacterial production, HNF = heterotrophic nanoflagellate abundance, POC = particulate organic carbon, TDCu = total dissolved copper. All the variables were measured daily except POC and TDCu that were measured at the beginning of the experiment (T_0), at the end (Tf), and, in the case of TDCu, after the aerosol addition (T_{AD}).

	SUMMER			**WINTER**				**SPRING**			
	C1	**A**	**S**	**C1**	**C2**	**A1**	**A2**	**C1**	**C2**	**A**	**S**
Inorganic nutrients, TOC	X	X	X	X	X	X	X	X	X	X	X
Chl *a*	X	X	X	X	X	X	X	X	X	X	X
Pico- and nanophytoplankton	X	X	X	X	X	X	X	X	X	X	X
HBA	X	X	X	X	X	X	X	X	X	X	X
HBP	X	X	X	-	X	X	X	-	X	X	X
HNF	X	X	X	-	X	X	X	-	X	X	X
POC (T_0, Tf)	X	X	X	-	X	X	X	-	X	X	X
TDCu (T_0, T_{AD}, Tf)	-	-	-	-	-	-	-	-	X	X	X

Table S3.2. Results of the ANCOVA performed for each measured variable in both C1 and C2 controls. The factors were CONTROL-TYPE, EXPERIMENT, and TIME (only the results for CONTROL-TYPE are shown). The number of samples (*N*), R^2 adjusted, and the *p*-value are detailed for each of the variables. *p<0.05; **p<0.01; ***p<0.001; n.s.: non-significant (p>0.05).

	N	***R^2* adj**	***p*-value**	**CONTROL-TYPE**
TIN	48	0.29	*	n.s.
TIP	48	0.69	***	n.s.
TOC	36	0.16	n.s	n.s.
Chl	42	0.74	***	n.s.
HBA	42	0.62	***	n.s.
Synechococcus	42	0.78	***	n.s.
Picoeukaryotes	42	0.73	***	n.s.
Nanoeukaryotes	42	0.57	***	n.s.

Table S3.3. Chemical composition of the collected filters used in the different experiments. A= anthropogenic, S= Saharan, TSP= total suspended particles, ME= major elements (expressed in µg m^{-3}), TM= trace metals (expressed in ng m^{-3}), <dl: below the detection limit. SD= standard deviation (only calculated when more than one A or S filters were mixed). The average and SD for major elements have been calculated for anthropogenic (A) and Saharan filters (S), presented as a percentage of TSP, as it is in this way that results are described in Section 3.3.1. *N:P calculated mol:mol.

	SUMMER				WINTER			SPRING		
ME (µg/m3)	**A**	**SD (A)**	**S**	**SD (S)**	**A1**	**SD (A1)**	**A2**	**A**	**SD (A)**	**S**
TSP	115.46	42.36	116.80	64.75	41.70	0.00	83.38	31.27	14.74	180.66
OC	10.18	3.04	7.10	1.81	7.13	1.05	17.34	5.91	2.15	9.59
EC	2.47	0.39	1.43	0.50	2.83	0.59	7.84	2.34	0.53	2.61
CO3	9.73	0.21	9.17	7.62	3.23	0.87	5.02	3.17	0.57	8.15
SiO2	35.52	19.63	42.07	40.45	2.14	0.39	5.16	4.24	2.33	50.36
Al2O3	11.84	6.54	14.00	13.47	0.71	0.13	1.72	1.41	0.78	16.79
Ca	6.48	0.16	6.13	5.12	2.15	0.58	3.34	2.11	0.38	5.43
Fe	4.23	1.78	4.50	4.26	1.15	0.15	2.30	1.11	0.21	5.96
K	2.81	0.78	3.00	2.70	0.52	0.41	0.67	0.47	0.30	2.63
Na	4.51	2.03	6.53	6.75	1.46	1.47	2.48	0.90	0.28	6.93
Mg	1.95	0.70	2.43	2.41	0.33	0.18	0.56	0.34	0.11	1.90
Cl-	3.81	1.55	6.20	8.50	2.08	2.56	3.40	1.00	0.22	9.49
SO42-	3.82	0.53	5.07	1.36	1.27	0.35	3.63	1.31	0.55	4.04
NO3-	4.92	0.92	4.43	1.63	2.54	1.19	9.91	2.46	2.39	3.82
NH4+	0.33	0.15	0.30	0.10	0.41	0.16	2.81	0.46	0.48	0.94
P	0.13	0.02	0.15	0.12	0.04	0.01	0.08	0.04	0.02	0.13
***N:P**	24.00		25.00		45.00		124.00	41.00		27.00

TM (ng/m3)	**A**	**SD (A)**	**S**	**SD (S)**	**A1**	**SD (A1)**	**A2**	**A**	**SD (A)**	**S**
Li	4.26	1.78	5.10	1.75	0.48	0.11	0.85	0.65	0.26	5.38
Be	0.16	0.07	0.20	0.07	0.05	0.00	0.07	0.05	0.02	0.34
B	<dl	<dl	<dl	<dl	14.07	12.50	<dl	<dl	< dl	<dl
Sc	1.19	0.6	1.34	0.42	0.08	0.01	0.16	0.15	0.07	1.21
Ti	332.36	143.53	374.30	133.52	44.10	11.69	125.91	64.62	29.31	781.31
V	24.50	4.81	30.84	13.93	6.10	4.39	18.92	9.50	7.58	19.03
Cr	37.70	3.46	51.06	24.21	14.19	7.47	16.25	8.25	4.43	15.78
Mn	109.51	48.77	122.97	42.95	24.33	0.55	36.84	25.97	1.65	109.14
Co	1.25	0.36	1.46	0.63	0.33	0.09	0.84	0.46	0.30	2.48
Ni	16.74	10.49	19.48	4.42	3.39	1.70	5.63	6.59	6.31	8.56
Cu	56.85	7.48	44.28	34.91	51.00	1.65	118.26	44.87	6.62	42.44
Zn	240.76	47.68	355.04	136.53	195.28	48.64	230.63	154.18	39.79	146.56
Ga	1.75	0.86	1.99	0.63	0.16	0.02	0.31	0.25	0.11	2.86
Ge	0.71	0.13	0.45	0.41	0.78	0.03	0.31	0.40	0.38	0.86
As	2.66	0.80	2.70	1.31	1.27	1.21	1.08	0.55	0.14	1.70
Se	0.74	0.23	0.67	0.36	0.27	0.25	0.57	0.33	0.33	0.44
Rb	11.71	5.77	12.70	4.20	0.97	0.32	1.74	1.40	0.73	11.80
Sr	27.98	9.94	32.27	12.75	5.60	1.79	8.35	6.13	1.94	38.06
Y	2.02	0.90	2.51	0.79	0.61	0.09	0.28	0.49	0.42	2.21
Zr	49.70	27.66	77.24	15.58	21.65	1.85	29.15	3.50	1.94	40.17
Nb	4.87	2.00	5.62	2.03	0.94	0.06	1.83	1.29	0.41	18.35

TM (ng/m3)	A	SD (A)	S	SD (S)	A1	SD (A1)	A2	A	SD (A)	S
Mo	12.57	1.66	27.25	7.71	26.12	3.11	8.37	9.70	12.50	18.73
Cd	0.27	0.13	0.22	0.10	0.25	0.16	0.42	0.24	0.05	0.25
Sn	10.04	2.42	5.36	5.39	7.77	0.29	16.52	6.80	3.56	5.94
Sb	5.28	0.38	3.34	3.46	5.03	0.15	12.16	4.07	1.12	8.02
Cs	0.56	0.27	0.59	0.21	0.09	0.02	0.14	0.12	0.07	0.63
Ba	175.00	123.89	338.24	36.14	93.06	10.81	127.07	56.76	56.75	147.89
La	3.47	1.59	3.93	1.33	0.51	0.14	0.76	0.65	0.29	5.23
Ce	6.28	2.98	7.09	2.33	0.99	0.15	1.64	1.26	0.53	11.72
Pr	0.76	0.37	0.87	0.28	0.08	0.01	0.13	0.12	0.06	1.37
Nd	2.73	1.33	3.13	0.99	0.29	0.04	0.42	0.44	0.20	4.81
Sm	0.62	0.30	0.73	0.23	0.08	0.02	0.08	0.10	0.06	1.00
Eu	0.11	0.05	0.13	0.04	0.02	0.00	0.03	0.02	0.02	0.19
Gd	0.60	0.30	0.74	0.21	0.11	0.02	0.08	0.12	0.08	0.90
Tb	0.09	0.04	0.12	0.03	0.02	0.00	0.02	0.02	0.02	0.14
Dy	0.52	0.25	0.68	0.19	0.15	0.04	0.06	0.11	0.09	0.67
Ho	0.10	0.05	0.12	0.03	0.02	0.01	0.01	0.02	0.02	0.14
Er	0.27	0.14	0.34	0.09	0.06	0.01	0.04	0.05	0.04	0.39
Tm	0.04	0.02	0.05	0.01	0.01	0.00	<dl	0.01	-	0.05
Yb	0.25	0.13	0.32	0.08	0.05	0.01	0.03	0.05	0.03	0.36
Lu	0.04	0.02	0.05	0.01	0.01	0.00	<dl	0.01	-	0.05
Hf	1.67	0.83	2.35	0.60	0.99	0.10	1.33	0.12	0.07	2.08
Ta	0.33	0.23	0.25	0.07	0.08	0.01	0.11	0.09	0.05	3.93
W	1.82	1.15	2.38	0.47	1.29	0.23	6.38	2.38	1.02	3.59
Tl	0.15	0.07	0.12	0.06	0.07	-	0.09	0.05	0.02	0.10
Pb	23.34	9.95	15.84	9.47	10.25	3.94	23.17	12.01	1.29	14.50
Bi	0.64	0.11	0.43	0.37	0.51	0.05	1.65	0.74	0.65	0.41
Th	1.47	0.75	1.81	0.51	0.24	0.05	0.24	0.21	0.13	1.06
U	0.48	0.24	0.82	0.17	0.34	0.06	0.14	0.14	0.11	0.63

	AVERAGE (% TSP)			
ME (µg/m3)	**A**	**SD (A)**	**S**	**SD (S)**
TSP				
OC	16.40	5.28	5.69	0.55
EC	6.45	3.08	1.34	0.15
CO3	8.08	1.70	6.18	2.36
SiO2	13.91	11.84	31.95	5.75
Al2O3	4.64	3.95	10.64	1.90
Ca	5.38	1.13	4.13	1.59
Fe	3.18	0.49	3.58	0.39
K	1.50	0.68	2.01	0.79
Na	3.31	0.48	4.72	1.24
Mg	1.05	0.46	1.57	0.73
Cl-	3.89	0.83	5.28	0.04
SO42-	3.72	0.64	3.29	1.49
NO3-	7.52	3.26	2.95	1.19
NH4+	1.53	1.32	0.39	0.19
P	0.11	0.02	0.10	0.04

Table S3.4. Mean values of element ratios and EF of certain metals in the collected filters. P:Al ratio and Fe:Al ratio were always above the threshold mineral ratios established by Guieu et al. in 2002 for dust (0.012 and 0.63, respectively) in the anthropogenic (A) aerosols and lower in the Saharan (S) ones, except in A filters collected during summer, which presented ratios slightly higher than the Saharan. Pb:Al and Cd:Al were quite above the threshold mineral ratios in all the filters (3.41×10^{-4} and 1.71×10^{-6}, respectively), but were lower in the Saharan ones. The Zn:Al ratio was always above the mineral ratio established by Guieu and Thomas in 1996 (1.01×10^{-3}) in all the filters but in the ones collected in summer. In good agreement with Nava et al. (2012), Si:Ca, Si:Fe, Al:Ca, Al:Fe, Ti:Ca, and Ti:Fe ratios were higher in S than in A. In contrast, Ca:Fe ratio was higher in the anthropogenic aerosols. The EF was always larger than one for all metals except in the case of titanium, which is a mainly crustal origin metal. Values were well above 10 for most metals in the anthropogenic filters, except in the summer one, in which the EF ratios were closer to the Saharan ones.

	SUMMER		WINTER		SPRING	
	A	S	A1	A2	A	S
P:Al	0.011	0.010	0.060	0.046	0.030	0.008
Fe:Al	0.357	0.321	1.608	1.340	0.782	0.355
Pb:Al	0.002	0.001	0.014	0.013	0.008	0.001
Cd:Al	2.25×10^{-5}	1.57×10^{-5}	3.49×10^{-4}	2.45×10^{-4}	1.73×10^{-4}	1.51×10^{-5}
Zn:Al	2.03×10^{-4}	2.54×10^{-4}	0.274	0.134	0.109	8.73×10^{-3}
Si:Ca	5.842	6.859	0.993	1.543	2.010	9.274
Si:Fe	8.401	9.348	1.866	2.239	3.837	8.449
Al:Ca	1.827	2.283	0.331	0.514	0.670	3.091
Al:Fe	2.800	3.111	0.622	0.746	1.279	2.816
Ca:Fe	1.532	1.363	1.880	1.451	1.909	0.911
Ti:Ca	0.051	0.061	0.020	0.038	0.031	0.144
Ti:Fe	0.079	0.083	0.039	0.055	0.058	0.131
EF_{Cu}	26.01	17.13	387.80	372.31	171.91	13.69
EF_{Ti}	0.70	0.66	1.54	1.82	1.14	1.16
EF_{Cd}	17.10	11.93	266.13	185.72	131.72	11.43
EF_{Pb}	8.98	5.15	65.56	61.37	38.69	3.93
EF_{Cr}	7.05	8.07	44.10	20.90	12.91	2.08
EF_{Zn}	30.29	37.77	408.38	199.68	162.43	13.00
EF_{Ni}	5.89	5.79	19.83	13.62	19.42	2.12
EF_{As}	8.70	7.47	68.96	24.23	15.01	3.91
EF_{Sn}	26.26	11.85	337.87	297.48	148.95	10.96

Table S3.5. Quotients of AIR from the biological variables divided by AIR of nutrients (mean ± standard deviation). SE= season; AE= aerosols.

	SUMMER		WINTER		SPRING	
	A	S	A1	A2	A	S
Chl/TIN	1.01 ± 0.43	1.10 ± 0.15	0.80 ± 0.05	0.40 ± 0.00	0.93 ± 0.08	1.39 ± 0.07
Chl/TIP	1.15 ± 0.16	0.88 ± 0.23	0.83 ± 0.01	1.07 ± 0.08	1.54 ± 0.15	1.35 ± 0.10
Syn/TIN	0.49 ± 0.25	0.56 ± 0.02	0.76 ± 0.06	0.37 ± 0.01	0.67 ± 0.00	1.15 ± 0.18
Syn/TIP	0.55 ± 0.02	0.45 ± 0.08	0.78 ± 0.01	0.99 ± 0.06	1.11 ± 0.00	1.12 ± 0.20
Pico/TIN	0.55 ± 0.29	0.56 ± 0.03	0.77 ± 0.05	0.38 ± 0.01	0.78 ± 0.15	1.27 ± 0.19
Pico/TIP	0.60 ± 0.01	0.45 ± 0.08	0.80 ± 0.01	1.02 ± 0.07	1.29 ± 0.26	1.24 ± 0.22
Nano/TIN	0.49 ± 0.27	0.55 ± 0.05	0.79 ± 0.05	0.40 ± 0.01	1.00 ± 0.06	1.23 ± 0.13
Nano/TIP	0.54 ± 0.01	0.44 ± 0.10	0.82 ± 0.00	1.09 ± 0.11	1.64 ± 0.12	1.20 ± 0.16
HBA/TIN	0.47 ± 0.23	0.52 ± 0.02	0.76 ± 0.05	0.37 ± 0.01	0.66 ± 0.04	1.20 ± 0.05
HBA/TIP	0.52 ± 0.03	0.42 ± 0.07	0.79 ± 0.00	1.01 ± 0.07	1.10 ± 0.07	1.17 ± 0.07
HBA/TOC	1.02 ± 0.10	1.07 ± 0.12	0.88 ± 0.00	0.89 ± 0.08	0.99 ± 0.04	1.01 ± 0.02
HBA/Chl	0.45 ± 0.04	0.48 ± 0.05	0.95 ± 0.00	0.94 ± 0.01	0.71 ± 0.02	0.87 ± 0.01
HBP/TIN	0.80 ± 0.26	0.75 ± 0.14	0.94 ± 0.14	0.38 ± 0.02	0.92 ± 0.01	1.35 ± 0.04
HBP/TIP	0.95 ± 0.24	0.61 ± 0.18	0.98 ± 0.20	1.03 ± 0.05	1.52 ± 0.00	1.32 ± 0.07
HBP/TOC	1.86 ± 0.56	1.53 ± 0.39	1.09 ± 0.23	0.91 ± 0.10	1.38 ± 0.03	1.14 ± 0.03
HBP/Chl	0.82 ± 0.09	0.68 ± 0.03	1.18 ± 0.25	0.96 ± 0.03	1.00 ± 0.10	0.97 ± 0.02
HNF/TIN	0.46 ± 0.23	0.48 ± 0.02	0.85 ± 0.01	0.40 ± 0.10	0.66 ± 0.01	1.19 ± 0.03
HNF/TIP	0.50 ± 0.02	0.38 ± 0.06	0.88 ± 0.06	1.06 ± 0.17	1.09 ± 0.03	1.16 ± 0.06

Chapter 4

The origin of atmospheric particles determines their effect on coastal bacterioplankton metabolism

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Abstract

In oligotrophic regions, such as the Mediterranean Sea, atmospheric deposition has the potential to stimulate prokaryotes growth and production in surface waters, especially during summer. Previous studies focused on the role of leaching nutrients from mineral particles of Saharan origin. In spite of increasing anthropogenic emissions to the atmosphere, the effects of these particles on marine bacterioplankton remain poorly assessed. In this study, we evaluate the effect of atmospheric particles of different origin and with a markedly different chemical composition (Saharan dust and anthropogenic aerosols) on marine planktonic communities from three locations of the north-western Mediterranean with contrasted anthropogenic footprint. Our results reveal an overall larger stimulation of heterotrophic production and of extracellular enzyme activities with anthropogenic particles, mainly attributed to their higher content of inorganic nitrogen by unit weight.

4.1 Introduction

Marine heterotrophic prokaryotes (hereafter referred to as "bacteria") play fundamental roles in the oceanic carbon cycle (Jiao et al., 2010). In oligotrophic regions, such as the Mediterranean Sea, most of the non-recalcitrant organic carbon (OC) is recycled in the surface layer and rapidly re-exchanged with the atmosphere (Moutin and Raimbault, 2002). This process is dependent on the availability of inorganic nutrients to bacteria inhabiting the surface waters, though. Large amounts of dissolved OC during the summer stratification period have been observed in Mediterranean surface waters, and attributed to a "malfunctioning" of the microbial loop, brought forth by nutrient limitation (mainly phosphorous, P, or P along with nitrogen, N) or competition between heterotrophic bacteria and phytoplankton (Pinhassi et al., 2006; Romera-Castillo et al., 2013; Thingstad et al., 1997). Instead, bacterioplankton growth and production in the Mediterranean has been found to be limited in OC during periods of winter mixing (Pinhassi et al., 2006; Thingstad et al., 1997), when nutrients are more abundant. The OC is a complex pool of molecules with different lability for bacterioplankton. Once the most labile fraction is exhausted, bacteria produce ectoenzymes in order to be able to hydrolyze polymeric substrates and then take up the monomers produced (Chróst, 1990).

The Mediterranean Sea is a quasi-enclosed basin, where external sources of nutrients have the potential to stimulate plankton growth and new production in surface waters. Changes in the relative importance of these external sources influence global oceanic C sequestration and affect CO_2 air–sea exchange (Bréviere et al., 2015). Among these sources, atmospheric deposition is considered an increasingly important source of new nutrients to the coastal ocean (Duce et al., 1991; Paerl et al., 2002). In oligotrophic

areas, such as the Mediterranean, the Sargasso Sea, or the north Atlantic, Saharan dust is known to play a fertilizing effect on marine planktonic microorganisms (both autotrophic and heterotrophic), especially during stratification conditions of the water column (Duarte et al., 2006; Gallisai et al., 2016, 2014; Herut et al., 2005; Laghdass et al., 2011; Mackey et al., 2012; Marañón et al., 2010). The chemical composition of Saharan dust over the Mediterranean atmosphere, although variable depending on the source area, has been broadly characterized, being the oxides of silicate and aluminum the main components (Escudero et al., 2011; Guieu et al., 2002; Moreno et al., 2006; Nava et al., 2012). In comparison, anthropogenic particles in the Mediterranean atmosphere are richer in N, sulfate, and organic compounds, as well as trace metals (Guerzoni et al., 1999; Moreno et al., 2013; Pateraki et al., 2012). In the northern hemisphere, the main sources of atmospheric N are anthropogenic (Guerzoni et al., 1999). With regard to P, anthropogenic inorganic emissions have been estimated to contribute as much as 50% to the total P deposition over the oligotrophic ocean (Christodoulaki et al., 2016). Furthermore, major elements and trace metals delivered from anthropogenic sources are much more soluble than those released by mineral dust due to their higher acidity, and thus more available for planktonic communities (Jickells and Moore, 2015; Migon et al., 2001; Nenes et al., 2011; Stockdale et al., 2016). However, in spite of the still high anthropogenic atmospheric emissions to the Mediterranean and at global scale (Duce et al., 2008; IPCC, 2014), their effect on marine bacteria remains poorly studied. In the Mediterranean Sea, a few studies have evaluated the effect of anthropogenic aerosols on bacterial abundance (Bonnet et al. 2005; Ternon et al. 2011), showing almost no response. Although a higher sensitivity of bacterial production to external changes compared to bacterial abundance has been observed (Gasol and Duarte 2000), in the Mediterranean, the effect of anthropogenic

aerosols on marine bacterial production, to our knowledge, has only been assessed in one location during the stratification period (Herut et al., 2016). On the other hand, Krom et al. (2016) tested the effect of Saharan dust on the activity of alkaline phosphatase (APA), an ectoenzyme mainly produced by algae to split phosphorous from organic matter, whereas, no studies are known on the effect of aerosols on other extracellular enzymes that are mainly synthesized by bacteria, as aminopeptidades and glucosidases (Sala et al., 2001, 2006b, and references therein). As extracellular enzyme activity constitute the initial response of a microbial community to environmental changes (Karner and Rassoulzadegan, 1995), their study may be useful to indicate the effect of aerosols on bacterial metabolism.

Moreover, bacteria may not directly take up nutrients from the aerosols but could be indirectly stimulated through phytoplankton growth. Previous aerosol-amendment experiments have focused their attention either on the effect of atmospheric particles on the bacterial community only, without competitors or predators (Pulido-Villena et al., 2008) or with phytoplankton and grazers altogether (Herut et al., 2005; Lekunberri et al., 2010; Pulido-Villena et al., 2014), being difficult to estimate the magnitude of the direct effect of atmospheric particles on bacteria. To properly assess the effect of both Saharan dust and anthropogenically-derived aerosols on bacteria, we performed a parallel experiment with and without phytoplankton and bacteria grazers.

Most of the studies that assess the effect of aerosols on the marine planktonic community are reduced to single locations or performed at a given time of the year. As in the Mediterranean Sea – and in most water bodies located in midlatitudes – the initial biogeochemical conditions in the seawater may differ among locations and times of the

year, we evaluated the effect of both types of atmospheric particles at two coastal locations of the NW Mediterranean with a different anthropogenic footprint (Barcelona, urban location, and Blanes, less impacted by human activities) and in open ocean waters offshore the Balearic Islands. We also explored the effect of aerosols at different times of the year in Barcelona. We hypothesize that i) a larger stimulation of bacterial growth and activity will occur with anthropogenic aerosols, due to their higher content in inorganic and organic soluble compounds; ii) a larger response of bacteria in the most oligotrophic location (offshore waters) is expected, as the initial nutrient concentration will be lower. For the same reason, a larger response is expected in late spring and summer than in winter in Barcelona; iii) bacterial growth and activity will be higher when no competitors and predators are present.

4.2 Methodology

4.2.1 Aerosol Collection

Total suspended particles (TSP) were sampled during dry atmospheric conditions (i.e. no rain) on quartz fiber filters (Munktell; Falun, Sweden). Particles were collected with a CAV-A/mb high volume sampler (MCV; Barcelona, Spain) operating at 30 m^3 h^{-1} during 24 h. The device was located at the Institut de Ciències del Mar of Barcelona (41.39º N, 2.20º E) and at the Centre d'Estudis Avançats of Blanes (41.68º N, 2.80º E). One half of each filter was used for chemical composition analyses, and the other half was employed for inoculation into the experimental containers. Atmospheric particles were recovered submerging the filters in 250 ml of artificial seawater (NaCl EMSURE, Grade ACS, MERCK; Darmstadt, Germany) and after sonication in a Bandelin SONOREX Digital 10 P Ultrasonic bath (MERCK; Darmstadt, Germany) at 7 kHz. Saharan ("S") and anthropogenic ("A") aerosols were classified based on advanced

event warnings for the north-eastern (NE) Iberian Peninsula (www.calima.ws), and the element ratios criteria defined in the literature (see Chapters 3 and 5 for a more detailed description of aerosol collection and classification criteria).

4.2.2 Experimental design and analytical procedures

Seawater collection and microcosm set-up. Water was collected ca. 1km from the coast of Barcelona (BCN) and Blanes (BLA), and offshore Balearic Islands (OFF), in the NW Mediterranean Sea (Fig. 4.1). Three experiments were carried out in Barcelona: in late summer of 2013, and in winter and spring of 2014. In Blanes, we perform two experiments, one in spring and other in summer of 2014, and the experiment offshore was carried out in late summer of 2014 (see Fig. 4.1 for an overview of the experimental set-up). Surface water sieved through a 150-µm-nylon mesh was collected in acid-cleaned carboys and taken to the laboratory at the Institut de Ciències del Mar within two hours. OFF instead was performed on board the R/V *García del Cid*. Seawater was distributed into 15-l acid-cleaned, methacrylate containers (BCN, BLA) or 10-l acid-cleaned, polypropylene carboys (OFF). Containers were incubated in a temperature-controlled chamber at the *in situ* surface water temperature and subjected to the corresponding photoperiod at that time of the year. Experiments lasted between 2 (OFF) to 6 days (BLA; spring) after the aerosol addition.

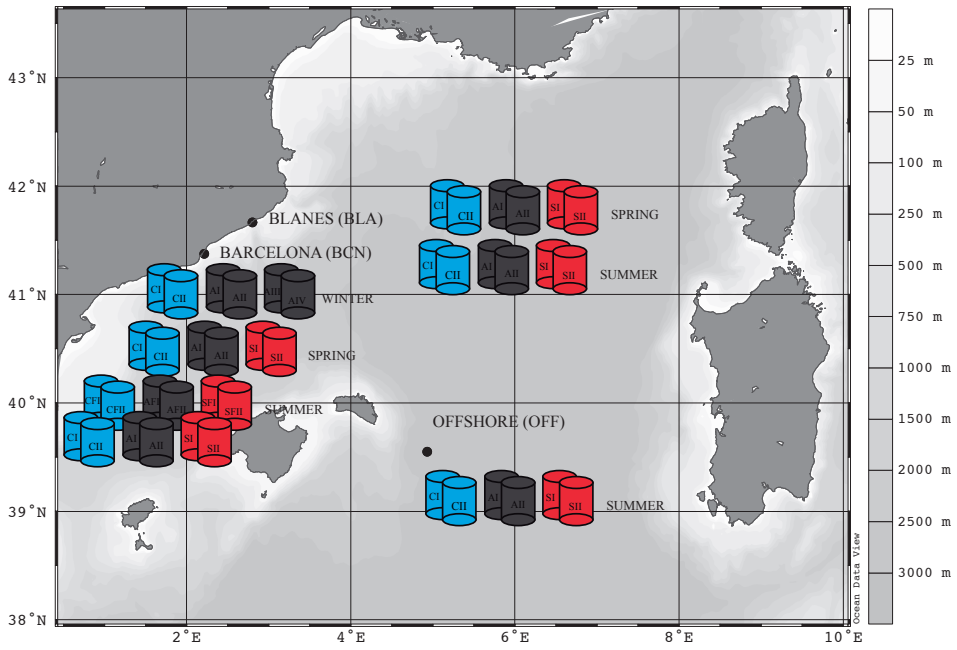

Figure 4.1. Geographical location of the two coastal sampling stations (Barcelona and Blanes), and the offshore station. A scheme of the experimental set-up is shown. The basic design consisted of two containers amended with Saharan dust (SI and SII), two with anthropogenic particles (AI and AII), and other two controls (CI and CII). In the winter experiment, there were no Saharan samples but two containers amended with one type of anthropogenic particles (AI and AII) and other two with anthropogenic particles of different composition (AIII and AIV). In the summer experiment of Barcelona, we used other 6 containers with seawater further filtered by 0.8 µm (CFI, CFII, AFI, AFII, SFI, SFII).

Aerosol additions. Atmospheric particles of anthropogenic origin (A) or Saharan dust (S) were added at 0.8 mg l^{-1} into duplicate experimental containers (A and S, respectively). The aerosols were added as unique doses and containers were subsequently stirred with a sterile glass stick to homogenize particle distribution. Blank treatments were carried out (C), containing either no particles or seawater with blank sonicated filters as in A and S. These two types of control showed no significant differences between them (see Tables S3.2 and S5.1). In the winter experiment instead, due to the higher frequency of pollution episodes of anthropogenic origin from Europe (Pey et al., 2010; Viana et al., 2005), we decided to have two anthropogenic aerosol treatments of different chemical composition. Aerosols used for each experiment were

collected at the corresponding location (i.e., BCN or BLA), with the exception of OFF. Here, aerosols from BCN were used for the amendment, as there was no time to previously collect the aerosols offshore. With the aim to evaluate the effect of aerosols on bacteria considering no grazers (mainly heterotrophic nanoflagellates) or competitors (phytoplankton), in the summer experiment in Barcelona, the same set-up was also carried out with seawater filtered by 0.8 μm to remove all cells but picoplankton, leaving basically heterotrophic bacteria and *Prochlorococcus* behind (treatments CF, AF and SF hereafter).

Biochemical sampling and analyses. Samples were taken from each microcosm by siphoning water through milli-Q rinsed and autoclaved glass tubes. Duplicate containers for each of the three treatments were simultaneously and independently sampled. Temperature was monitored daily using a digital thermometer VWR 8202-156 (VWR International; Radnor, PA, USA). Sampling for inorganic nutrients (nitrate, nitrite, ammonium, phosphate and silicate), total organic phosphorous (TOP), total organic carbon (TOC), heterotrophic bacterial abundance (HBA), heterotrophic bacterial production (HBP), and enzymatic activity was performed daily. Analyses of fluorescent dissolved organic matter (FDOM) were carried out before and after the aerosol additions, in order to assess the quality of the organic matter supplied by both types of atmospheric particles. Biolog plates were used at the beginning and at the end of the experiments to estimate the potential utilization of sole carbon sources released by the aerosols.

Inorganic nutrients, TOC, HBA and HBP were measured following standard procedures that can be found elsewhere (see Chap. 3 for a detailed description). Briefly, 10 ml-

samples of inorganic nutrients were measured with an Auto Analyzer AA3 HR (SEAL Analytical; Norderstedt, Germany) following the methods described in Grasshoff et al. (1999). For TOC determination, 10 ml-samples were collected in pre-combusted glass ampoules (450 ºC, 24 h), acidified to pH < 2 and stored in the dark until analyzed with a TOC-5000 analyzer (SHIMADZU; Kioto, Japan) following the high temperature catalytic oxidation (HTCO) technique described by Cauwet (1994). HBA was determined by flow cytometry using a Becton Dickinson FACScalibur flow cytometer (Becton Dickinson; Franklin Lakes, NJ, USA) with a laser emitting at 488 nm (Gasol and del Giorgio, 2000). HBP was measured using the [^{3}H] leucine (leu) incorporation technique (Kirchman et al., 1985) with the modifications of Smith and Azam (1992). Conversion of leu incorporation to bacterial production was done assuming a 0.073% of leu in protein and a C:P of 0.86 (Simon and Azam, 1989), using an isotope dilution factor of 2 typical for oligotrophic water and bacterial cell volumes determined by flow cytometry. Specific HBP was calculated dividing bulk HBP by the bacterial abundance.

TOP was determined by extracting inorganic phosphorous to total phosphorous. Total phosphorous was determined following the wet oxidation and colorimetric analysis described by Hansen and Koroleff (1999). Samples of 25 ml were mixed with 2 ml of the oxidant reagent in glass vials and autoclaved at 121 °C for 30 min. Once the vials were cooled down to room temperature, 556 µl of ascorbic acid and the same amount of the combined reagent were added. The samples were kept in the dark for 15 min before analysis. Readings were done at 880 nm with a CaryWin UV Spectrophotometer (VARIAN; Palo Alto, CA, USA).

Samples for FDOM determination were analyzed after temperature acclimation according to Nieto-Cid et al. (2006). Single measurements of seawater and emission-excitation (Ex/Em) matrices were performed with a Perkin Elmer luminescence spectrometer LS-55 (Waltham, MA, USA). Measurements were performed in a 1 cm quartz cell. The Ex/Em matrices were generated by concatenating 21 synchronous spectra over excitation wavelengths of 250 to 450 nm and emission wavelengths of 300 to 650 nm. The Ex/Em wavelengths used for single measurements were those described by Coble (1996): Peak-C (Ex/Em 340 nm/440 nm) as indicator of terrestrial-like substances, Peak-M (320 nm/410 nm) as indicator of marine-like substances and Peak-T (280 nm/420 nm) as indicator of protein-like substances.

The activities of three extracellular enzymes were determined using the fluorogenic substrates (all from Sigma-Aldrich Co. Ltd, MERCK; Darmstadt, Germany): $_{L}$-leucine-7-amido-4-methyl-coumarin, 4-methylumbelliferone-phosphate, and 4-methylumbelliferone-β-D-glucoside for the enzymes leu-aminopeptidase (AMA), alkaline phosphatase (APA), and ß-glucosidase (ß-Gl), respectively. Subsamples (315 µl) and substrates (final concentration 125 µM) were added to black 96 microtiter-well-plates in 4 replicates. Fluorescence was measured at t_0, immediately after addition of the substrate, and after incubations of approximately 15 min, 1h, 3h and 5h in an Infinite M200 spectrofluorometer (Tecan; Männedorf, Switzerland) or a Modulus microplate reader (Turner BioSystems; Sunnyvale, CA, USA) at 365 nm emission and 450 nm excitation wavelengths. Incubations were performed inside the temperature-controlled chamber. The increase of fluorescence units during the incubation was converted into activity by preparing a standard curve with the end product of the reactions 7-amino-4-methyl-coumarin for AMA, and 4-methylumbelliferone for APA and ß-Gl. Specific

AMA and ß-Gl, two enzymes synthesized mainly by heterotrophic bacteria (Sala et al., 2016, 2001), were calculated dividing bulk AMA and ß-Gl by the bacterial abundance.

To determine the potential utilization of sole carbon sources, Biolog-GN™ microplates (MERCK; Darmstadt, Germany) were used, following a similar procedure to that in Sala et al. (2006). Biolog-GN are 96-well microtiter plates containing 95 different carbon sources and one blank. With the oxidation of the carbon sources, redox dye tetrazolium violet is reduced to formazan that can be quantified photometrically. Each well was inoculated with 150 µl of sample water and then the plates were incubated at room temperature. After incubation, absorbance was measured using a spectrophotometric microplate reader (ELX800 BioTek Instruments at 590 nm; Winooski, VT, USA). For the statistical analyses, carbon sources were grouped in amines, amides, amino acids, carbohydrates, glucosamines, phosphorilated-sugars, nucleosides and histidines.

4.2.3 Statistical analysis

We evaluated the effect of aerosols on nutrient dynamics by subtracting their concentration at the sampling time before the addition to that at the sampling time after the addition. Then nonparametric Wilcoxon tests (Wilcoxon, 1945) were conducted to look for differences between treatments (C, A, S). We also carried out Wilcoxon tests to evaluate the response of the biogeochemical variables to different conditions during the incubation period. Data were normalized to initial experimental values. We tested the experiments with the same characteristics: 1) the effect of the TREATMENT (C, A, S) was assessed considering all ("ALL" hereafter) the experiments but the winter one (as there were no S samples in this experiment); 2) differences between TREATMENT and

LOCATION (BCN, BLA, and OFF) were tested for the three summer experiments; 3) differences between SEASON (winter, spring and summer) were tested in Barcelona; 4) differences between TREATMENT and the FILTRATION process (samples filtered by 0.8 µm with respect to those not filtered) were tested in the summer experiment in Barcelona ("SUBCN"). Samples filtered by 0.8 µm were only considered for the last analysis. Finally, to analyze the correlation between the chemical and the biological response in the two types aerosols, we calculated the AIR values for each variable (as explained in Sect. 3.2.5 of Chap. 3) and then the nonparametric Spearman's ρ correlation coefficient for each pair of response variables. Three separate tests were performed: once for AIR values in the A samples (all data except the filtered samples), another for the S samples (all data except the filtered samples), and a specific test for the SUBCN samples. For the last analysis we considered all the samples of this experiment (filtered and unfiltered). Significance was considered for $p < 0.05$. Analyses were performed with JMP (SAS) version 10.0 and R (v 3.2.4).

4.3 Results

4.3.1 Effect of aerosols on nutrient dynamic

After the aerosol additions, all nutrients increased their concentration in the amended microcosms (A, S) – on average –, except nitrite (NO_2^-), that slightly decreased (Fig. 4.2). The decrease was lower than that observed in the controls, though. The variability among experiments was high, especially in the case of TOC. Overall, statistical analysis showed that the concentration of ammonium (NH_4^+), silicate, and TOC significantly increased in the A microcosms compared to the C after the addition ($p = 0.043$, $p = 0.0481$ and $p = 0.038$, respectively). The addition of nitrate (NO_3^-) was statistically higher in the A than in both the C and the S containers ($p = 0.0043$ and $p = 0.0118$,

respectively), resulting in a significant increase of total inorganic nitrogen (TIN) in the A compared to the other treatments. Total inorganic and organic phosphorous (TIP and TOP, respectively) instead only showed a moderate increase in the A and the S, on average.

Considering the whole incubation period, NO_3^- was also significantly higher in the A containers than in the S and the C, and in the S than in the C (Table 4.1). TIN was significantly higher in both the A and the S than in the C for all the situations tested, and also in the A than in the S when considering all the experiments except the winter one (*ALL*; Table 4.1; see sect. 4.2.3). For this situation, NH_4^+, TIP, and TOC were significantly higher in the A than in the C, and TOC was also significantly higher in the A than in the S. Considering the summer experiment of BCN in particular, TIP was statistically higher in both amended microcosms than in the controls. No significant differences between treatments were found for TOP in any of the comparison tested.

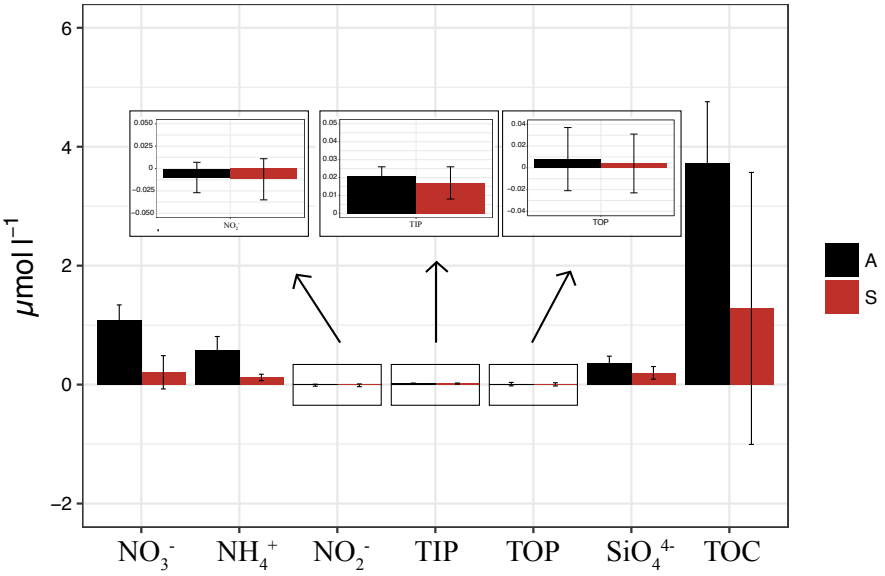

Figure 4.2. Difference in the concentration (µmol l^{-1}) of NO_3^-, NO_2^-, NH_4^+, TIP, TOP, SiO_4^{4-}, and TOC after the aerosol additions - with respect the values approx. 3h before the addition - in the experimental microcosms (A and S). Error bars represent the standard error from replicate containers in the six experiments.

Table 4.1. Results of the Wilcoxon test for the different comparison assessed. 'sp' refers to specific rates of HBP, AMA and ß-Gl, respectively. TREAT = TREATMENT (C, A, S); FILT = filtration (F = samples filtered by 0.8 µm; NF = not filtered); WI= winter; SP= spring; SU= summer. n.s.: non-significant ($p > 0.05$); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. n.d.: not determined (TOP was not measured in the filtered samples). Light grey highlights the variables for which significant differences were found between the A and the other two treatments simultaneously.

	ALL	***SUMMER***		***BARCELONA***	***SUBCN***	
	TREAT	TREAT	LOCATION	SEASON	TREAT	FILT
NO_3^-	***(A>C) ***(A>S) ***(S>C)	***(A>C) *(A>S) ***(S>C)	***(OFF>BCN) **(OFF>BLA) **(BLA>BCN)	***(WI>SU) ***(WI>SP)	***(A>C) **(A>S) **(S>C)	n.s.
NH_4^+	**(A>C)	n.s.	***(OFF>BLA) *(BCN>BLA)	***(WI>SP) ***(SU>SP)	n.s.	n.s.
TIN	***(A>C) ***(A>S) ***(S>C)	***(A>C) ***(S>C)	***(OFF>BCN) **(OFF>BLA)	***(WI>SP) ***(SU>SP)	**(A>C) *(S>C)	***(NF>F)
TIP	*(A>C)	n.s.	***(OFF>BCN) **(OFF>BLA)	n.s.	***(A>C) ***(S>C)	n.s.
TOP	n.s.	n.s.	**(BCN>OFF) **(OFF>BLA) ***(BCN>BLA)	***(SU>WI) *(SP>WI)	n.s.	n.d.
TOC	**(A>C) *(A>S)	n.s.	**(BCN>OFF) **(BCN>BLA)	***(SU>WI) ***(SP>WI)	n.s.	n.s.
HBA	n.s.	n.s.	**(BCN>OFF) ***(BCN>BLA)	***(SU>WI) ***(SP>WI)	n.s.	***(F>NF)
HBP	*(A>C)	*(A>C)	***(OFF>BCN) ***(OFF>BLA) ***(BCN>BLA)	***(SP>WI) ***(SP>SU)	***(A>C) ***(S>C)	***(F>NF)
HBP (sp)	**(A>C)	*(A>C)	***(OFF>BCN) ***(OFF>BLA)	***(WI>SU) ***(SP>WI) ***(SP>SU)	***(A>C) **(S>C)	**(F>NF)
AMA	**(A>C) *(A>S)	n.s.	***(BCN>BLA)	n.s.	n.s.	***(F>NF)
AMA (sp)	***(A>C) *(A>S)	n.s.	***(BCN>BLA)	***(WI>SU) ***(WI>SP)	*(A>C)	***(F>NF)
ß-Gl	***(A>C) *(A>S)	*(A>C)	*(BCN>OFF)	***(WI>SU) ***(WI>SP)	*(A>C) *(S>C)	***(F>NF)
ß-Gl (sp)	***(A>C)	n.s.	*(BLA>OFF)	***(WI>SU) ***(WI>SP)	*(A>C)	***(F>NF)
APA	n.s.	n.s.	**(OFF>BCN)	***(WI>SU)	n.s.	***(F>NF)

	***(BLA>BCN)	***(WI>SP) **(SP>SU)

Regarding the organic compounds, FDOM measurements showed an increase in humic-like and protein-like substances after the aerosol additions in the amended microcosms, except in the filtered samples of Barcelona (Fig. 4.3). Analysis of carbon substrates using Biolog-ECO microplates showed statistical differences between treatments for the group of carboxylic acids, which were more used in the A microcosms than in the controls (*p* value = 0.059).

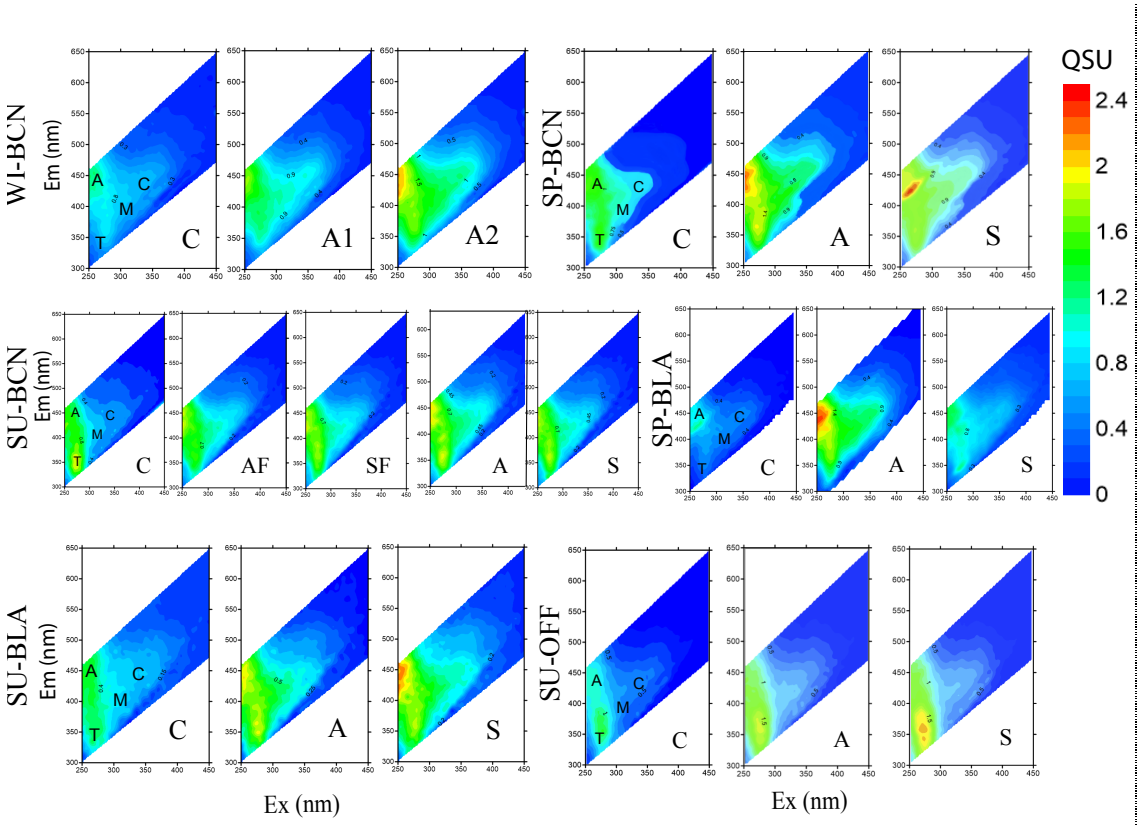

Figure 4.3. Changes in the excitation (Ex)-emission(Em) matrix of FDOM after the aerosols addition in the six experiments. Florescence before the addition in all the microcosm – expressed in quinine sulfate units (QSU) – is the same than in the controls (C), whereas A and S matrixes show the values after the addition of aerosols. The different peaks are indicated.

4.3.2 Bacterial activity

The effect of atmospheric particles on bacterial abundance was moderate and dependent on the location. Bacterial abundance was more stimulated with S in Barcelona and offshore, whereas the response was higher with A in Blanes (Fig. 4.4). Instead, aerosols, especially of anthropogenic origin, produced a large enhancement on bacterial production (Fig. 4.5) and extracellular enzymatic activity (Figs. 4.6, 4.7 and 4.8). As the effect on bacterial abundance was overall lower, specific (sp) production and activities (sp AMA and ß-Gl) were still higher in the A containers than in the S and the C. On the other hand, in Barcelona, in the summer experiment, all the biological variables showed a high response in the A and S filtered samples (AF and SF) compared with the filtered controls (CF).

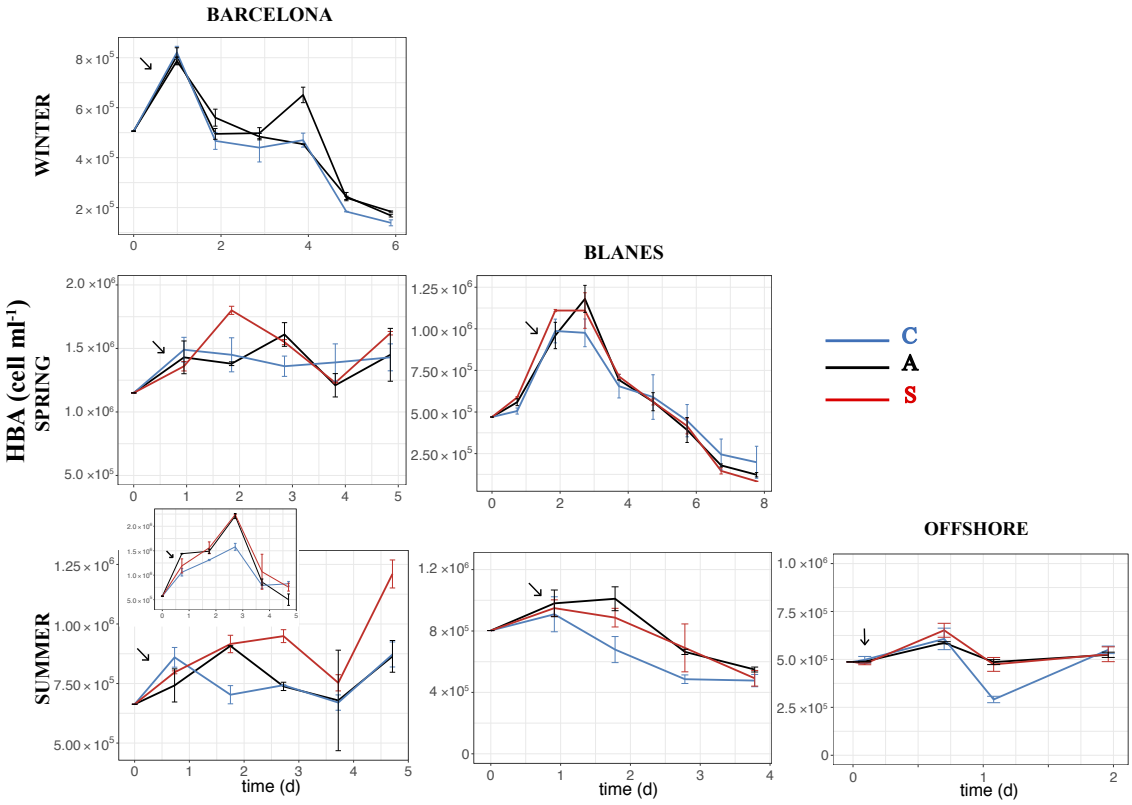

Figure 4.4. Heterotrophic bacterial abundance – HBA (cell ml^{-1}) – over the incubation time in the six experiments. Error bars represent the standard error from two replicate containers. Arrows point the moment of aerosol additions. C = controls; A = anthropogenic; S = Saharan. The plot in the upper left corner of summer Barcelona corresponds to the filtered samples (CF, AF, SF). Note that in the winter

experiment there are two anthropogenic treatments.

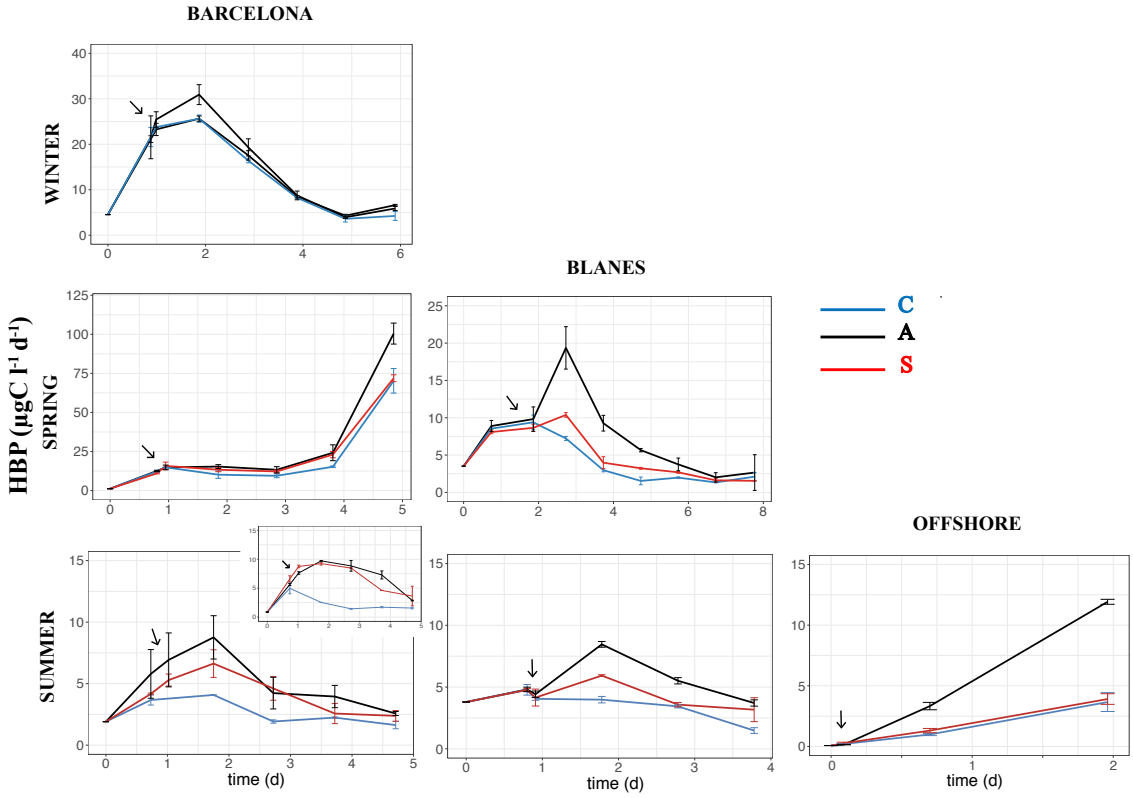

Figure 4.5. Heterotrophic bacterial production – HBP (µg C l^{-1} d^{-1}) – over the incubation time in the six experiments. Error bars represent the standard error from two replicate containers. Arrows point the moment of aerosol additions. C = controls; A = anthropogenic; S = Saharan. The plot in the upper right corner of summer Barcelona corresponds to the filtered samples (CF, AF, SF; same scale as in the non-filtered samples and the other summer experiments). Note that in the winter experiment there are two anthropogenic treatments.

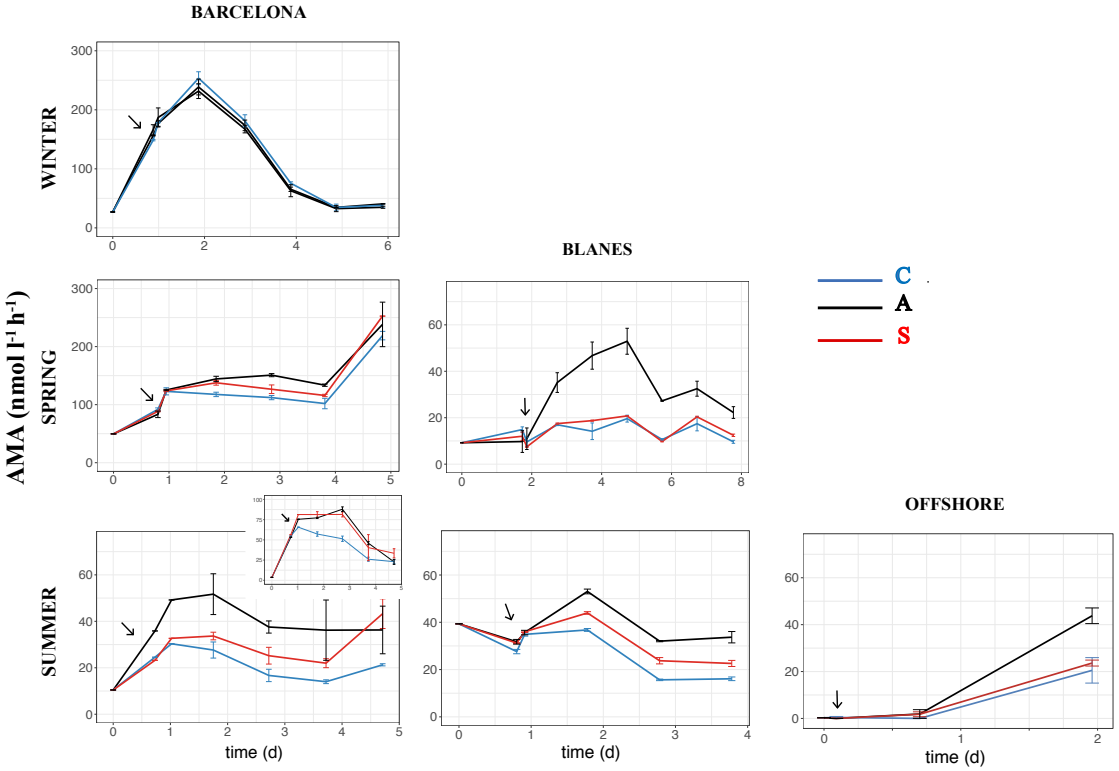

Figure 4.6. Activity of leu-aminopeptidase – AMA (nmol l^{-1} h^{-1}) – over the incubation time in the six experiments. Error bars represent the standard error from two replicate containers. Arrows point the moment of aerosol additions. C = controls; A = anthropogenic; S = Saharan. The plot in the upper right corner of summer Barcelona corresponds to the filtered samples (CF, AF, SF; scale: from 0 to 100). Note that in the winter experiment there are two anthropogenic treatments.

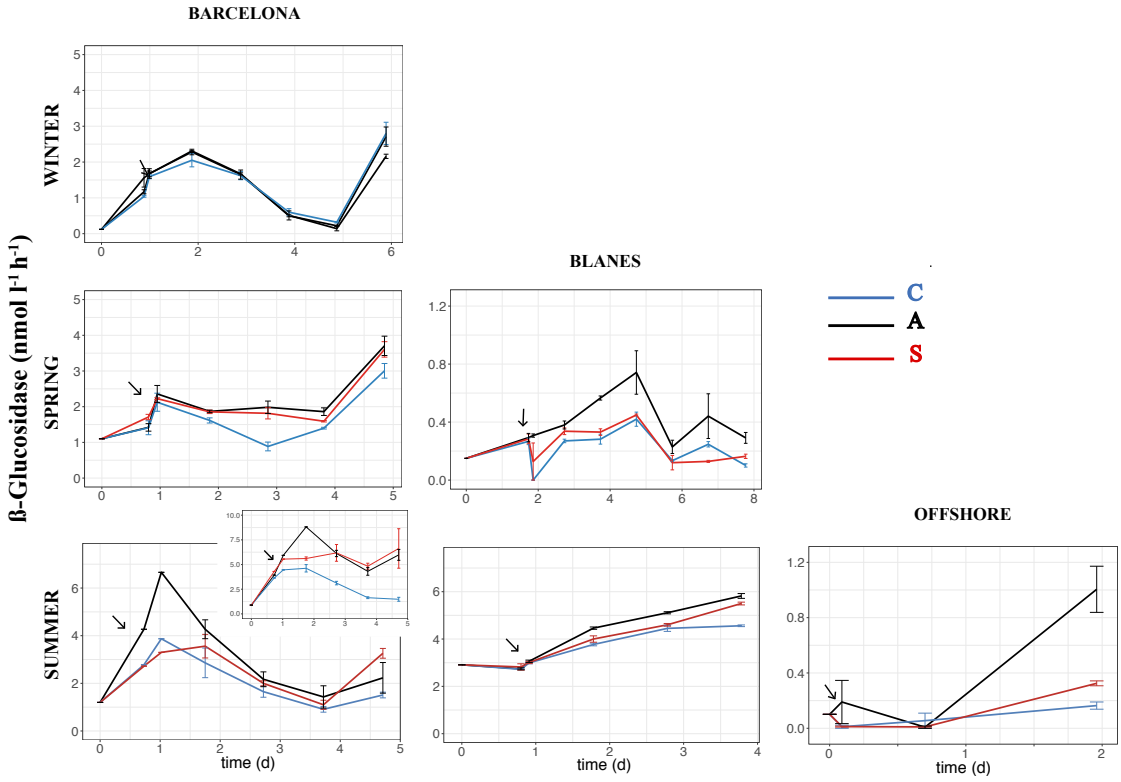

Figure 4.7. Activity of ß-Glucosidase (nmol l^{-1} h^{-1}) over the incubation time in the six experiments. Error bars represent the standard error from two replicate containers. Arrows point the moment of aerosol additions. C = controls; A = anthropogenic; S = Saharan. The plot in the upper right corner of summer Barcelona corresponds to the filtered samples (CF, AF, SF; scale: from 0 to 10). Note that in the winter experiment there are two anthropogenic treatments.

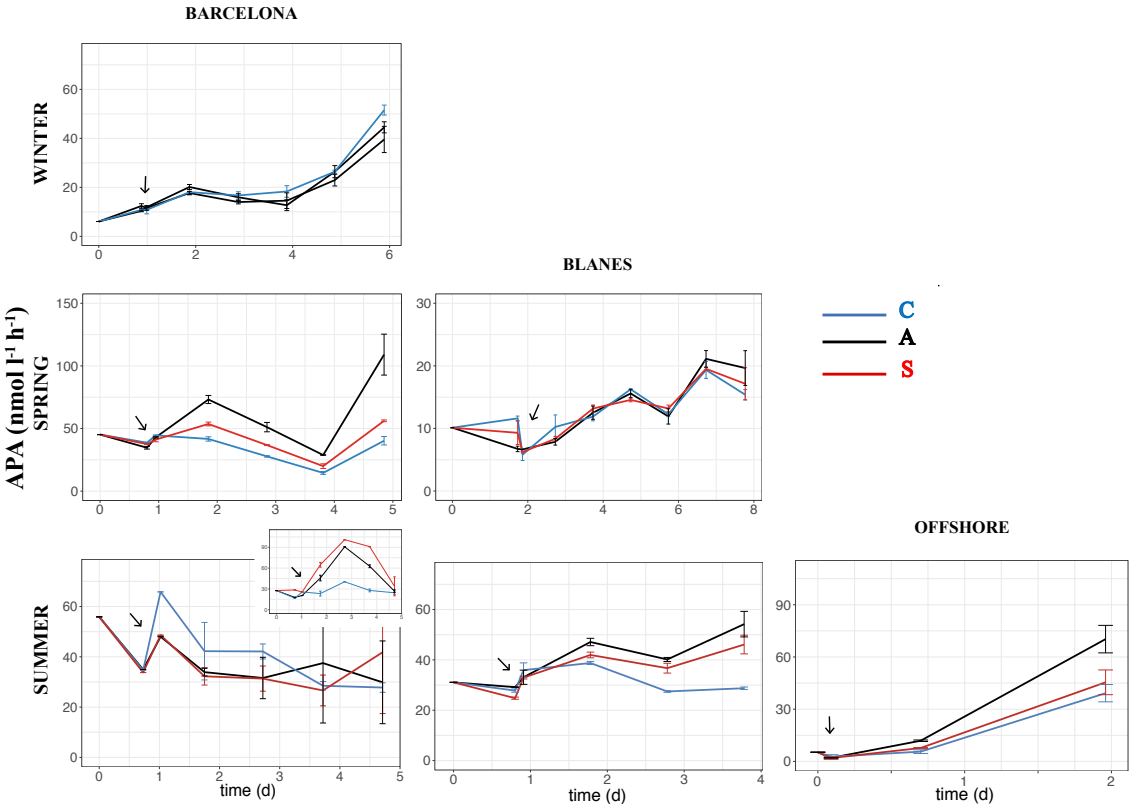

Figure 4.8. Activity of alkaline phosphatase – APA (nmol l^{-1} h^{-1}) – over the incubation time in the six experiments. Error bars represent the standard error from two replicate containers. Arrows point the moment of aerosol additions. C = controls; A = anthropogenic; S = Saharan. The plot in the upper right corner of summer Barcelona corresponds to the filtered samples (CF, AF, SF; same scale as in the OFFSHORE experiment). Note that in the winter experiment there are two anthropogenic treatments of different composition.

4.3.2.1 Effect of aerosols at different locations and times of the year

The effect of atmospheric particles on seawater was statistically assessed comparing 1) the results from all the experiments but the winter one (*ALL*), 2) the three locations in summer (*SUMMER*), and 3) the filtration in *SUBCN* (Table 4.1). In the first comparison, AMA, spAMA, and ß-Gl, were significantly higher in the A than in the other microcosms, and HBP, spHBP, and spß-Gl, were statistically higher in the A than in the C. Comparing the three experiments carried out in summer, statistical differences were found for HBP, spHBP and ß-Gl, which showed higher values in the A microcosms than in the C (Table 4.1). With respect to the location, HBP and spHBP

were significantly more stimulated offshore than in Barcelona and Blanes, and APA was higher offshore and in Blanes than in Barcelona. HBA, AMA, spAMA, ß-Gl, and spß-Gl instead were statistically higher in Barcelona. Inorganic nutrients were overall significantly higher offshore than in the coastal locations, while TOP and TOC were statistically higher in Barcelona. Regarding the effect of the season, assessed for the three experiments in Barcelona, HBA and HBP were significantly larger in spring and summer than in winter, while enzymatic activities were higher in winter than in the other seasons. Inorganic nitrogen was higher in winter than in the other seasons, whereas TOP and TOC were larger in summer and spring than in winter.

To investigate the overall relationship between the elements released by the aerosols and the response in the biological variables, we calculated the Spearman's ρ coefficient for each pair of variables in the A and the S microcosms (Fig. 4.9). In the A samples, the biological variables showed a high positive correlation among them and with NO_3^- and TIN. In the S samples instead, correlations were moderate. In this case, we did not found significant correlations between any of the biological variables and N, but TIP showed a significant correlation with HBP and spHBP, and TOP with spHBP.

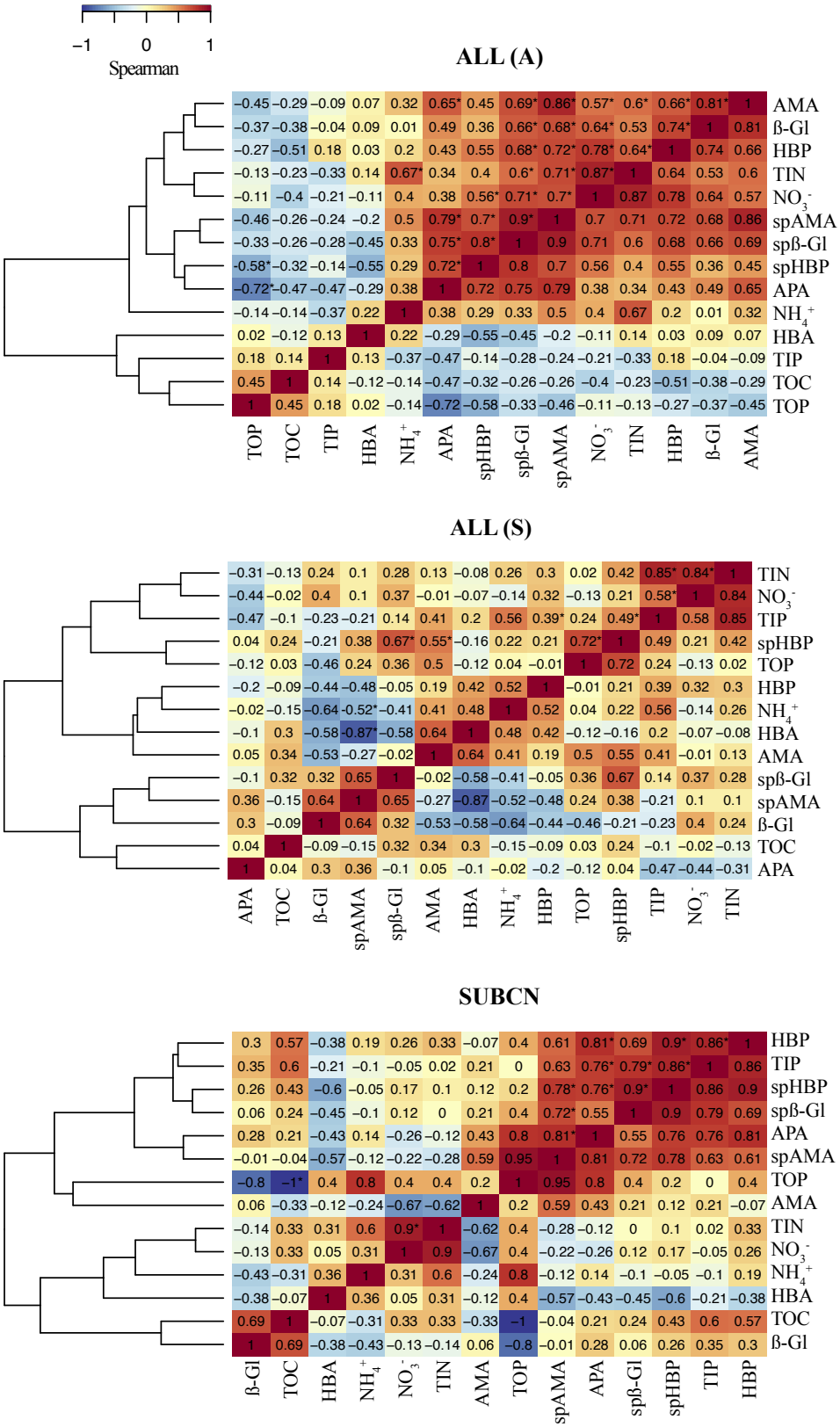

Figure 4.9. Heat map showing the Spearman's ρ coefficient between the response variables in the Anthropogenic and the Saharan samples (all data except the filtered samples). The correlation was also tested for the experiment of summer Barcelona (SUBCN). Significant correlations ($p < 0.05$) are indicated with an asterisk (*) in the upper left part of the symmetric matrix.

4.3.2.2 Direct effect of aerosols on bacteria

In the summer experiment of Barcelona (*SUBCN*), the effect of aerosols on bacterial abundance and activity was evaluated with and without competitors and predators, further filtering the seawater by 0.8 µm in 6 of the 15 l containers (section 4.2.2). Statistical analyses showed that HBP, spHBP, and ß-Gl were statistically higher in both amended microcosms than in the controls, and spAMA and spß-Gl were significantly more stimulated in the A containers than in the C (Table 4.1). All the biological variables were significantly more stimulated in the containers filtered by 0.8 µm than in those unfiltered. In this particular experiment, considering the filtered and unfiltered samples altogether, most of the biological variables showed a high positive correlation with TIP, which was significant in the case of HBP, spHBP, spß-Gl and APA (Fig. 4.9).

4.4 Discussion

4.4.1 Nutrients added with the aerosol inputs

Nutrient measurements one hour after the aerosol additions showed that Saharan dust released on average 0.25 ± 1.01 µmol TIN mg^{-1}, 0.02 ± 0.02 µmol TIP mg^{-1} and 1.67 ± 6.91 µmol TOC mg^{-1}. These values are within the reported values of TIN dissolved from Saharan dust in previous experiments carried out in the Mediterranean (Herut et al., 2016, 2005; Lekunberri et al., 2010). TIP concentration is higher than those usually found in the literature but within the range reported by Pulido-Villena et al. (2008) after a real deposition event of Saharan dust (2.58 g m^{-2}) in the NW Mediterranean (release of P: 0.03 µmol mg^{-1}), and in microcosm experiments adding similar concentrations of dust than in our experiments (0.02 - 0.03 µmol mg^{-1}). A high standard deviation was determined for TOC, but our values were within the ranges reported in previous studies (Duarte et al., 2006; Lekunberri et al., 2010; Pulido-Villena et al., 2008). Anthropogenic

particles released higher amounts of TIN (1.98 ± 1.79 µmol TIN mg^{-1}) and TOC (4.70 ± 3.45 µmol TIN mg^{-1}), but the same concentration of TIP per mg of aerosol. TIN released by these aerosols was slightly higher than that reported by Guo et al. (2014) (1 µmol mg^{-1}), but much lower than that determined by Bonnet et al. (2005) and Duarte (2006) (~ 24 µmol mg^{-1}). The similar concentration of P released from both types of particles, higher than expected for Saharan particles, must be related to the fact that Saharan particles arriving to the NE of the Iberian Peninsula are mixed with local and regional sources of pollution during their trajectory. As pollution involves increases in NOx and SOx compounds, the main acid precursors species in the atmosphere (Stockdale et al. 2016), and the solubility of P increases with acidity (Nenes et al., 2011; Stockdale et al., 2016), it seems reasonable to find a higher release of P from dust collected in the Catalan coast than in other experiments performed close to the desert and in more remote areas less exposed to pollution (e.g., Bonnet et al., 2005; Guo et al., 2016; Herut et al., 2005). In fact, Stockdale et al. (2016) suggested that the flux of bioavailable P from acidified dust is expected to increase in the Mediterranean Sea during the coming years. Another reason that can explain differences in nutrient release among published data is related to the high variability of the chemical composition of atmospheric particles themselves, depending on their source and the physico-chemical processes that they suffer in the atmosphere (Jickells and Moore, 2015; Law et al., 2013; Moreno et al., 2006). Moreover, there is also a variability induced by using different methodologies for aerosol collection and extraction, as discussed in Chap. 3.

The additions of TOC released with both aerosols (from 0 to 9 µmol per mg) are negligible compared to ambient concentration, that range from 50 to ca. 150 µmol l^{-1} in surface waters of the Catalan coast (Romera-Castillo et al., 2013; Vila-Reixach et al.,

2012). P released by the atmospheric particles is in the same range of P annual concentration in the offshore station (Lazzari et al., 2016; see Table 1.2 in Chap.1), and within the range of concentrations observed during the summer stratification period in Barcelona and Blanes (Arin et al., 2013; Guadayol et al., 2009b; Romero et al., 2014), both aerosols having the potential to be an important source of P during this period. TIN released by Saharan dust was overall small compared to ambient concentrations in the western Mediterranean, but it might also be an important source of N during the stratification period, when TIN in the seawater can be < 0.1 µM (Arin et al., 2013; Guadayol et al., 2009b; Romero et al., 2014). The average TIN concentration leached by the anthropogenic particles (ca. 2 µmol per mg of aerosol) is equivalent to the annual average concentration in Blanes and in the offshore station, and ca. 8 times higher than that during the stratification period (Gasol et al., 2012; Lazzari et al., 2016). This concentration is also equivalent to the summer average in Barcelona (Arin et al., 2013; Romero et al., 2014). Anthropogenic aerosols have therefore the potential to constitute an important source of bioavailable N during the whole year in coastal locations of the NW Mediterranean, but especially during the stratification period.

4.4.2 Aerosol effects on bacterial abundance and metabolism

Our results show that heterotrophic bacterial production was significantly enhanced with both types of atmospheric particles (in the summer experiment of Barcelona), or only with the anthropogenic (when all the experiments were considered altogether), whereas aerosols did not yield significant increases in bacterial abundance. These results are in agreement with previous studies carried out with Saharan dust, where a great stimulation of bacterial production was observed, in comparison with no or lower response on bacterial abundance (Duarte et al., 2006; Lekunberri et al., 2010; Marañón

et al., 2010; Pulido-Villena et al., 2014; Ridame et al., 2001). Studies that evaluated the effect of anthropogenic aerosols on bacterial abundance did not observe significant increases either (Bonnet et al. 2005; Ternon et al. 2011), while Herut et al. (2016) also observed a larger increase in bacterial production in the eastern Mediterranean after the addition of anthropogenic aerosols. Teira et al. (2013) performed addition experiments with rainwater added to coastal eutrophic waters of the Ría de Vigo (NW Spain) and found also a higher effect on bacterial production than on abundance, too.

We also looked at the activity of three extracellular enzymatic activities: AMA, APA and ß-Glucosidase. Ectoenzymes can be produced by a large number of microorganisms, such as heterotrophic nanoflagellates, zooplankton, algae and bacteria (Karner et al., 1994; Jamet and Boge, 1998). Nonetheless, APA is believed to be mainly synthesized by algae, whereas AMA and ß-Glucosidase would be mainly produced by bacteria (Vives-Rego et al., 1985, Münster et al. 1992). Thus, the enzymatic activities related with heterotrophic bacteria were the ones more stimulated with anthropogenic aerosols (AMA, specific AMA, ß-Gl, and specific ß-Gl). An increase in AMA activity is indicative of either TIN limitation or a supply of dissolved organic nitrogen, while an increase in ß-Glucosidase points to a higher hydrolysis of carbohydrates, mainly derived from cellulose (Karner and Rassoulzadegan, 1995; Misic et al., 2008; Rath et al., 1993; Sala et al., 2001, 2006b). As anthropogenic particles produced a significant increase in inorganic N (and in the N:P ratio), we can discard the stimulation of AMA as indicative of TIN limitation. Hence, the observed enhancement of AMA and ß-Gl activities determined in these experiments in the A microcosms must be attributed to an addition of organic sources of N and C.

In aerosol-amended experiments where the whole planktonic community is assessed, bacteria might take up the organic compounds needed for growth either from the atmospheric particles or from those released by phytoplankton cells. The concomitant increase of HBP and the three enzymatic activities observed towards the end of the experiment carried out in Barcelona in spring (Figs. 4.5 – 4.8) may indicate a release of organic compounds from phytoplankton cells, as a peak of chlorophyll (Chl) was observed 1 day after the aerosol additions, and then phytoplankton concentration quickly decreased towards the end of the experiment, and so decreased TIN and TIP (see Chap. 3). However, the enhancement of HBP and enzymatic activities observed in Blanes during spring and in the summer experiments is unlikely an indirect effect from phytoplankton released compounds, as HBP and enzymatic activities started to increase earlier or at the same time than Chl in these experiments (Annex I shows the dynamics of Chl in all the experiments). Aerosols, especially of anthropogenic origin, were also a source of TOC. More specifically, the enhancement of ß-Gl and the higher availability found for carboxylic acids with anthropogenic aerosols, suggest that carbohydrates and carboxylic acid compounds may be a source of OC derived from anthropogenic particles. The stimulation of AMA also suggests a supply of organic nitrogen. Thus, although, we did not find a correlation between the TOC added with the aerosols and the biological response (Fig. 4.9) − probably because the concentration was low compared to that initially present in the seawater −, the stimulation of AMA and ß-Gl points to the exhaustion of the labile fraction of organic compounds in the seawater (Chróst, 1990) and the hydrolysis of polymeric substrates that either were already present or come from the aerosols. On the other hand, Kerner et al. (1992) found that the enhancement of AMA and ß-Gl in marine snow was much higher than the increase in organic pools, what explained by the stimulation of different bacterial groups within

marine snow. In our experiments, we observed a preferentially stimulation of *Alphaproteobacteria* (mainly from the order *Rhodobacterales*) and *Gammaproteobacteria* (order *Alteromonadales*) with the anthropogenic particles (see Chap. 5).

Regarding the inorganic nutrients, we found that anthropogenic aerosols released significantly more nitrate and total N than Saharan dust. Hence, the higher stimulation of HBP and enzymatic activities observed with A points to N (and more specifically nitrate) as the major driver in the observed response. In fact, considering all the results from the six experiments, most of the biological variables showed a high correlation with nitrate and TIN in the A samples (Fig. 4.9). The response to Saharan dust instead was only significant for HBP and spHBP in the experiment of *SUBCN* (Table 4.1), and probably more related to the supply of P, as a significant correlation was found between these variables and TIP, and with TOP for spHBP (Fig. 4.9). It must be noticed that the concentration of P related to that of N at the beginning of *SUBCN* was much lower than in the other experiments (where N:P ratio was < 16). Hence, the overall increase observed in HBP and enzymatic activities with the atmospheric (especially with the anthropogenic) particles should be a combination of three different factors: (1) the supply of inorganic nutrients (i.e. TIN and TIP) and some bioavailable organic compounds from the aerosols, (2) the re-mineralization of particulate organic matter already present in the seawater, and (3) a change in bacterial assemblages favoring the growth of more active bacteria. In agreement with this hypothesis, Guo et al. (2016) found an increase in the active fraction of *Alphaproteobacteria* just 3h after the addition of aerosols in the eastern Mediterranean, whereas *Gammaproteobacteria* were enhanced later on, presumably following the increase in phytoplankton biomass.

When evaluating the effect of the location in summer, a larger stimulation of HBP and APA was observed offshore than in the coastal locations. Marañón et al. (2010) showed that the effect of aerosols on HBP increased with increasing oligotrophy. Hence, the larger increase of HBP observed offshore can be attributed to the higher oligotrophy of the open ocean waters offshore Balearic Islands (Chl annual average of 0.25 ± 0.09 μg l^{-1}; Gallisai et al. 2014) in comparison with the coastal waters of Barcelona (Chl average of 1.58 ± 1.09 μg l^{-1}; Romero et al. 2014) and Blanes (Chl average of 0.63 ± 0.05 μg l^{-1}; Guadayol et al. 2009). Furthermore, inorganic N and P were also significantly higher offshore. In contrast, HBA, AMA, and ß-Gl were more stimulated in Barcelona. TOC and TOP were significantly higher in Barcelona, but is difficult to establish causality, as both remained at similar concentrations in all the microcosm through the incubation time. With respect to the season, the effect of aerosols on HBA and HBP was higher in summer and spring than in winter, which can also be attributed to the lower concentration of inorganic nutrients and organic matter in the seawater at this time of the year in Barcelona (see Table 3.1 in Chap. 3). Enzymatic activity instead was higher in winter than in the other seasons, but without apparent differences between treatments (Figs. 4.6 - 4.8). In this experiment, an increase in Chl was observed the day after the addition, followed by a decrease toward the end of the experiment (Fig. 3.3 and Annex I). Thus, the major stimulation of enzymatic activities observed in winter in all the microcosms is likely due to the remineralization of organic compounds already present in the seawater.

Finally, we have shown that bacterial abundance and activity can be directly stimulated by atmospheric deposition, and that the effect is higher without the presence of

competitors (i.e. phytoplankton) and predators (i.e. heterotrophic nanoflagellates). These results explain why previous studies where only bacteria were considered (e.g., Pulido-Villena et al., 2008) showed a higher effect of Saharan dust on bacterial abundance with respect to other experiments where the whole planktonic community was considered, at similar dust additions (e.g., Herut et al., 2005). Furthermore, the effect of both types of atmospheric particles on almost all the biological variables was similar in the filtered samples, except ß-Gl and specific ß-Gl, which were higher in anthropogenic microcosms (Figs. 4.4 - 4.8, SUMMER-BARCELONA). These observations suggest that, in the absence of competitors, bacteria show a similar preference to both types of aerosols. Then, the higher bacterial production and activity of AMA observed with the anthropogenic particles when the whole community was evaluated must be attributed to either the indirect stimulation after phytoplankton growth with these particles (which may be the case of Barcelona during spring) or to the presence of other competitors for Saharan dust. For instance, cyanobacteria were significantly more stimulated with Saharan dust than with the other treatments in the experiment carried out in Blanes during summer (Chap. 5). And, particularly in the summer experiment of Barcelona, we found that several taxa belonging to the groups *Alphaproteobacteria* and *Flavobacteria* increased in the filtered samples amended with both aerosols, whereas *Cyanobacteria* decreased. The enhancement of *Synechococcus* with Saharan dust has already been observed in other experiments carried out in the Mediterranean (Giovagnetti et al., 2013; Herut et al., 2005; Pitta et al., 2017).

All the same, directly or through an indirect stimulation mediated by phytoplankton growth, our results show that heterotrophic bacteria production and enzymatic activity are significantly more stimulated with anthropogenic than with Saharan aerosols, as

they provide more inorganic (mainly N) and organic nutrients to the seawater. In agreement, Herut et al. (2016) found a larger stimulation of bacterial abundance and activity in response to anthropogenic atmospheric inputs than with Saharan dust in the eastern Mediterranean. It must be pointed that, as anthropogenic aerosols are a major source of inorganic N than P, these particles are expected to produce a higher impact on the heterotrophic community when N is a co-limiting nutrient, as we observed at the beginning of most of the experiments (N:P ratios < 16). Instead, in the summer experiment of Barcelona, when the initial N:P in the seawater was quite above the Redfield ratio of 16, the response of the biological variables was mainly triggered by P, as pointed by the higher correlations between the biological variables and P observed particularly in this experiment (Fig. 4.9). Thus, although P is thought to be the main limiting nutrient in the Mediterranean during the whole year (Lazzari et al., 2016; Marty et al., 2002; Sala et al., 2002), anthropogenic aerosols have a huge potential to stimulate bacterial activity when both N and P are in low concentrations, a common situation in coastal (Guadayol et al., 2009a) and open waters (Moutin et al., 2012) of the western Mediterranean during the stratification period. Furthermore, N is the main limiting nutrient in most of the oligotrophic ocean (Moore et al., 2013). As these areas represent more than 50% of the global ocean (Antoine et al., 1996), it is important to address the effect of an increasingly polluted and acidified atmosphere (IPCC, 2014; Stockdale et al., 2016) in oligotrophic aquatic regions.

4.5 Conclusions

In the present study we show that anthropogenic particles produce a larger effect on bacterial production and enzymatic activity than Saharan dust at the same particle concentration. This is attributed to the major release of bioavailable inorganic (mainly

N) and organic compounds. When the initial status of the seawater was poor in inorganic nutrients but rich in organic carbon, bacteria outcompeted phytoplankton in uptaking the inorganic nutrients released by aerosols, whereas other times the effect on bacteria was mediated through a previous stimulation of phytoplankton. When no competitors and predators are present, both anthropogenic and Saharan atmospheric particles yield a major enhancement of bacterial abundance and activity.

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Chapter 5

Atmospheric deposition shapes bacterial community assembly in the northwestern Mediterranean

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Abstract

Atmospheric deposition is an important source of nutrients to the oligotrophic ocean, with the potential of stimulating bacterial growth and production. While the impact of mineral dust (mainly from the Saharan desert) on bacterial community composition (BCC) has been previously assessed, only one study is known in which the effect of anthropogenic aerosols on marine bacterial assemblages has been assessed up to date. Here, we assess the effect of atmospheric particles of different origin (i.e., Saharan dust and anthropogenic aerosols) on marine bacterioplankton community composition across a spatial and temporal range of trophic conditions in the northwestern Mediterranean Sea. Results from 16S rDNA pyrosequencing showed that seasonality and geographical location were the main determinants of the composition of bacterial assemblages. Atmospheric deposition yielded significant changes in community composition at certain places and during certain times of the year, which accounted for shifts in the bacterial community of up to 48%. Significant differences in the bacterial community composition were also found between the two types of atmospheric particles assessed. Anthropogenic particles favored more the growth of *Alphaproteobacteria* and *Bacteroidetes* (*Flavobacteria* and *Sphingobacteria*), whereas Saharan dust stimulated preferentially *Cyanobacteria* (mainly *Synechococcus*).

5.1. Introduction

Marine bacterioplankton plays a fundamental role in the cycling of carbon (C) via the microbial carbon pump (Jiao et al., 2010). In the ocean, there are photosynthetic bacteria (i.e. *Cyanobacteria*) that take up CO_2 and transform it in organic matter, and heterotrophic bacteria, which either transform labile dissolved organic carbon (OC) into recalcitrant OC that persists for a very long time in the ocean, or remineralize parts of the OC and thus release CO_2 back into the atmosphere. While micro-organisms modify the composition of the OC pool, the composition of organic matter compounds and their availability to microbes strongly influence bacterioplankton activity and phylogenetic diversity (Jiao and Zheng, 2011; Kirchman et al., 2004; Logue et al., 2015). Apart from OC, there are other factors that control community structure, as the bioavailability of inorganic nutrients that may limit bacterial growth and production (Kirchman et al., 2004).

In the oligotrophic Mediterranean Sea, as in the global ocean, large amounts of OC have been recorded in its surface waters during summer stratification (May - October). This phenomenon has been attributed to a "malfunctioning" of the microbial loop, brought forth by nutrient limitation and/or competition between prokaryotes and phytoplankton (Pinhassi et al., 2006; Romera-Castillo et al., 2013; Thingstad et al., 1997). As a matter of fact, Mediterranean waters - especially during that stratification period - are depleted of inorganic nutrients. Hence, the growth and production of the marine planktonic community is predominantly limited by phosphorus (P) or P along with nitrogen (N); the latter co-limitation found mainly in the northwestern (NW) basin (Guadayol et al., 2009; Pulido-Villena et al., 2014; Sala et al., 2006). Nutrient limitation is more conspicuous in the open ocean than in coastal waters, which is illustrated by a mean

annual chlorophyll concentration of ca. 0.25 $\mu g l^{-1}$ (Gallisai et al., 2014; Gomes et al., 2015), as to 0.63 and 1.5 observed in waters adjoining rural and urban areas of the Catalan coast, respectively (Arin et al., 2013; Guadayol et al., 2009; Romero et al., 2014). On the other hand, the Mediterranean has been found to be limited in OC during periods of winter mixing (December - March) either in open (Thingstad et al., 1997) or coastal (Pinhassi et al., 2006) waters, thereby also restricting heterotrophic planktonic growth and production.

The deposition of particles from the atmosphere onto the Mediterranean Sea may, to some extend, alleviate the nutrient and OC shortage. The atmosphere of the NW Mediterranean experiences frequent inputs of total suspended particles (TSP) from European and local sources but also frequent dust events from the Saharan desert (Guerzoni et al., 1999; Rodríguez, 2002). With deposition fluxes of TSP of up to thousand times greater (up to 22 g m^{-2} d^{-1}) (Ternon et al., 2010) than during normal weather conditions (e.g., 15 mg m^{-2} d^{-1} in the eastern coast of Spain; Pulido-Villena et al., 2008), Saharan dust events can account for a large part of atmospheric inputs in the NW Mediterranean, especially during summer (Querol et al., 2009). In addition, large variations in atmospheric deposition background levels have been observed between Mediterranean waters bordering urban (39 μg m^{-3}; Barcelona) (Pey et al., 2008) and rural (16 μg m^{-3}; Montseny atmospheric station, located at the north-east of Spain) (Querol et al., 2009) areas. The chemical composition of Saharan dust over the Mediterranean atmosphere, although variable depending on the source area, has been broadly characterized, with the main chemical constituents being silicate and aluminum oxides (Moreno et al., 2006; Nava et al., 2012). To a lower extent, Saharan dust is also a source of N, P, OC, and metals to land and ocean (Guerzoni et al., 1999; Morales-

Baquero et al., 2006; Ridame and Guieu, 2002). While N is thought to be soluble in seawater (and thus bioavailable), the fraction of leachable P from dust is highly variable (7–100%) (Nenes et al., 2011; Stockdale et al., 2016). On the other hand, anthropogenic aerosols are more acidic compared to Saharan dust, which is why its P, organic compounds, and trace metals are much more soluble than Saharan dust in seawater (Durrieu de Madron et al., 2011; Longo et al., 2014), and therefore more bioavailable to the plankton.

Marine bacterioplankton have been shown to capitalize on both mineral and anthropogenically-derived aerosols (Martínez-García et al., 2015; Pulido-Villena et al., 2008; Herut et al., 2016). However, their effect on the bacterial community composition (BCC) remains poorly studied, most of the previous work focused on the effect of Saharan dust and based on molecular techniques of low taxonomic resolution as fluorescence in situ hybridization (FISH) or denaturing gradient gel electrophoresis (DGGE) (Laghdass et al., 2012; Lekunberri et al., 2010; Marañón et al., 2010; Pulido-Villena et al., 2014; Reche et al., 2009). Only two recent studies have assessed the effect of anthropogenic (mixed with mineral particles) aerosols on the bacterial community (Guo et al., 2016; Rahav et al., 2016), but they were restricted to a single location at a given time of the year (during spring, offshore Crete). Results from these studies suggest that dust deposition can selectively stimulate certain bacterial groups by providing specific conditions favorable to their growth. Hence, as each specific taxon or clade has its own growth requirements, variations in the proportion of nutrients supplied by aerosols (both mineral and anthropogenically-derived) may imply major changes in the composition of bacterioplankton assemblages. And because the concentration of both types of aerosols are expected to increase globally due to desertification and

human activities (Duce et al., 2008; Durrieu de Madron et al., 2011; Reche et al., 2009), studying their effect on marine bacterial assemblages is of great global interest. In this study, we assess the relationship between atmospheric deposition of aerosols of different origin and BCC in the NW Mediterranean Sea by means of 16S rDNA pyrosequencing. To do so, we carried out six different experiments covering a spatial and a temporal gradient: in summer, we tested the effect of aerosols in three locations that differed in their anthropogenic signature (coastal waters of a urban location, another coastal area less affected by human impacts, and offshore waters), and sampling was done from summer to winter (in the urban location). In each experiment, we tested the effect of Saharan dust and anthropogenic aerosols compared to non-amendment conditions. We hypothesize that aerosols will favor the growth of certain bacterial groups over others depending on the initial conditions, as the inorganic nutrients and OC concentration in the seawater would be different. We hypothesize that i) anthropogenic particles will yield a larger stimulation of heterotrophic bacteria, due to their higher content in soluble P and organic compounds. ii) bacterial community structure of the urban location, especially in winter conditions, will be less disturbed that in more oligotrophic conditions/locations, as bacteria may benefit from other nutrient sources.

5.2. Methods

5.2.1 Aerosol collection

TSP were sampled at the Institut de Ciències del Mar of Barcelona (41.39º N, 2.20º E) and at the Centre d'Estudis Avançats of Blanes (41.68º N, 2.80º E), on days that Saharan particles arrived to the north-eastern (NE) Iberian Peninsula ("S" aerosols), or during normal meteorological conditions ("A" aerosols). TSP were, thereby, collected

on quartz fiber filters (Munktell; Falun, Sweden) by means of a MCV CAV-A/mb high volume sampler (30 m^3 h^{-1}, 24 h) (MCV; Barcelona, Spain). Saharan dust and anthropogenic aerosols were classified based on advanced event warnings for the NE Iberian Peninsula (www.calima.ws), and the element ratios criteria defined in the bibliography (Guieu et al., 2010; Migon et al., 2001; Nava et al., 2012; Wedepohl, 1995). Each filter served two purposes: one half was used for chemical composition analyses, while the other was employed as inoculum in the subsequent aerosol-addition experiments (see Supplementary Methods S5.1 for more detail).

5.2.2 Experimental set-up

Water was collected either close to the coast of Barcelona (BCN) and Blanes (BLA), or offshore Balearic Islands (OFF), in the NW Mediterranean Sea. Experiments were carried out in late summer of 2013 in Barcelona (SUBCN), and during 2014 in winter and spring in Barcelona (WIBCN and SPBCN), spring and summer in Blanes (SPBLA and SUBLA), and in summer offshore (SUOFF) (Table 5.1, see also Fig. 4.1 in Chap. 4 for an overview of the experimental set-up). Surface water (0 – 50 cm) was collected in acid-cleaned carboys and, in the case of BCN and BLA, transferred to the laboratory in less than 2h. The SUOFF experiment was performed on board of the research vessel *García del Cid*. Once collected, the water was on site first sieved through a 150-µm-nylon mesh to remove macro-zooplankton and then distributed into 15-l acid-cleaned, methacrylate containers (BCN, BLA) or 10-l acid-cleaned, polypropylene carboys (OFF). Microcosms were incubated at *in situ* temperatures and submitted to *in situ* light cycles (see Table 5.1 for detailed information). Light intensity inside the containers was 225 µmol photons m^{-2} s^{-1}, which is approximately the saturating irradiance reported at the Catalan coast for most of the year (Guadayol et al., 2009).

Table 5.1. Summary of the experimental set-up. SST = sea surface temperature. The atmospheric treatment was evaluated using two duplicate containers amended with the same type of aerosols: C = control, A = anthropogenic, S = Saharan. TIME of collection refers whether the samples for BCC were collected at an intermediate time during the experiment (TM), or at the end of the incubation period (TF). FILTRATION indicates whether the samples were filtered by 150 µm (*in situ*) or further filtered by 0.8 µm.

	WIBCN	**SPBCN**	**SUBCN**	**SPBLA**	**SUBLA**	**SUOFF**
Date	25.02.2014	12.05.2014	18.09.2013	8.04.2014	30.06.2014	26.09.2014
SEASON	Winter	Spring	Summer	Spring	Summer	Summer
LOCATION	Barcelona	Barcelona	Barcelona	Blanes	Blanes	Offshore (Balearic Islands)
Latitude (ºN)	41.38	41.38	41.38	41.67	41.67	39.55
Longitude (ºE)	2.22	2.22	2.22	2.80	2.80	4.93
Depth (m)	40	40	40	22	22	2655
SST (ºC)	13.3	17.6	23.4	14.0	21.4	22.0
Light:dark (h)	11:13	14.5:09.5	12.5:11.5	13:11	15:09	12.5:11.5
TREATMENT	C, A	C, A, S	C, A, S	C, A, S	C, A, S	C, A, S
Days since the addition	5.38	4.36	4.38	6.32	3.38	1.9
TIME of collection	TM, TF	TM, TF	TF	TM, TF	TF	TF
FILTRATION (µm)	150	150	150, 0.8	150	150	150
Total # Samples	11	11	12	12	6	6

Aerosol amendments to the experimental containers consisted of 0.8 mg l^{-1} of either Saharan dust (S) or aerosols of anthropogenic origin (A). Amendments of 0.8 mg l^{-1} are equivalent to a medium-high Saharan deposition event of 8 mg m^{-2} (a value within the values of Saharan dust events reported in the NE coast of Spain) into a mixed layer

water column of 10 m, which is approximately the depth of the thermocline during the stratification period in the NW Mediterranean (D'Ortenzio et al., 2005). Another two containers were either not amended or amended with a blank filter processed as in A and S to verify that no fertilization effect could be attributed to the filters themselves. An analysis of covariance (ANCOVA) using the incubation time as covariate showed non-significant differences for any of the biogeochemical variables measured in the two types of controls but on *Synechococcus* (Table S5.1). Thus, to simplify, we consider them duplicates and refer them as controls (C).

In all the experiments, each treatment was evaluated in duplicate containers ($N = 2$). In SUBCN, the same set-up was also carried out with seawater pre-filtered by 0.8 µm to remove all cells but picoplankton, leaving basically only heterotrophic bacteria behind (CF, AF and SF), with a total of 12 containers in this experiment (Table 5.1). We also collected 12 samples for BCC determination in WIBCN, SPBCN and SPBLA, as we sampled at an intermediate time (TM) during the incubation period, and at the end of the experiments (TF). However, we lost one of the replicates from the C and another from the A samples in WIBCN and SPBCN, respectively, leaving 11 samples for each experiment. As Saharan events over the NW Mediterranean are less frequent during winter, we used four containers amended with 0.8 mg l^{-1} of anthropogenic-origin aerosols in WIBCN. Aerosols used for each experiment were collected at the correspondent location (i.e., BCN or BLA), with the exception of the experiment carried out offshore: here, aerosols from BCN were used for the amendment, as the campaign was not long enough to collect the filters on board.

5.2.3 Bacterial community composition (BCC)

Nucleic acid collection and extraction. Samples (250 ml) for BCC determination were filtered onto 0.2-µm polycarbonate filters (Durapore Membrane Filters, Millipore; Billerica, MA, USA). Filters were placed into sterile 2-ml cryogenic vials (Nalgene; Rochester, MN, USA) and stored immediately at -80 ºC. Bacterial DNA extraction was conducted following the procedure described in Schauer et al. (2000) including minor modifications. In brief, filters were submerged in lysis buffer (40 mM EDTA, 50 mM Tris [pH=8.3], 0.75 M sucrose) and - after lysozyme had been added at a final concentration of 1 mg ml^{-1} - incubated at 37 ºC for at least 45 min under slight movement. Upon adding proteinase K (0.2 mg ml^{-1} final concentration) and sodium dodecyl sulfate (10% v/v), samples were again incubated at 55 ºC for at least 1h. Approximately 750 µl of lysate was extracted from the filters, mixed twice with 750 µl of a mixture of phenol-chloroform-isoamyl alcohol (25:24:1, pH = 8), respectively, and once with 750 µl of chloroform-isoamyl alcohol (24:1). The aqueous phase was recovered and concentrated into Amicon Ultra-15 Centrifugal Filter Units (Millipore; Billerica, MA, USA) to approximately 250 µl, using a Sigma 3-16KL centrifuge (Sigma; Osterode am Harz, Germany) operating at 3000 rpm, and washed three times with 2 ml of supra-pure filtered (0.2 µm) water (milli-Q) to a final volume of 100 - 200 µl. The final extract was kept at -80ºC until further processing.

PCR amplification and pyrosequencing. PCR amplification and pyrosequencing were carried out at the Research and Testing Laboratory of Lubbock (http://rtlgenomics.com/ ; Texas, USA). See Supplementary Methods S5.2 for an in-detailed description hereof.

Sequence analyses. Pyrosequences were analyzed in QIIME (v 1.6) (Caporaso et al., 2011), following a similar procedure to that described by Pernice et al. (2015). After de-multiplexing and a first quality check, sequences were between 125 and 600 bp long, showed a quality score >25, contained no more than 2 mismatches in the primer sequences, and no homopolymers longer than 6 bp. To correct for reading mistakes, a DeNoiser algorithm (Reeder and Knight, 2009) was run only including the sequences that had passed the initial quality check. Denoised centroids and singletons were clustered into operational taxonomic units (OTUs) at a sequence identity level of 97% using UCLUST (Edgar, 2010). Prior to chimera detection and removal (ChimeraSlayer; Haas et al., 2011), representative sequences were aligned according to the SILVA (Quast et al., 2013) alignment (release 108) using MOTHUR (v 1.33.3) (Schloss et al., 2009). The remaining sequences were again aligned and taxonomy was assigned according to the SILVA alignment (release 123). OTUs were assigned to a given group in case its representative sequence showed a BLAST hit to a reference sequence with an e-value below 10^{-5}. OTUs with an e-value above this threshold were classified as uncertain. Finally, pyrosequences that were either assigned as Archaea, Eukaryota, or uncertain, and contained fewer than 2 reads, were removed from the final dataset, which consisted of 367,294 pyrosequences clustering into 3,434 OTUs. Sequence data have been submitted to the GenBank database under accession numbers SAMN05914882-SAMN05914941.

5.2.4 Statistical analyses

We used multivariate statistics to evaluate the relationship between BCC and the environmental variables considered in the study (SEASON, LOCATION, TREATMENT, TIME, and FILTRATION). Variance inflation factors were calculated

online at GUSTAME (Buttigieg and Ramette, 2014) to check that there was no co-linearity among the environmental variables ($p > 0.05$).

Alpha-diversity in the samples was calculated by means of the Simpson index (Eq. 5.1), which considers the combined richness and evenness within communities (Oksanen et al., 2008). The number of reads per sample was previously normalized by dividing the total number of reads in each sample by the lowest number of reads in the matrix. In order to compare samples from experiments with the same characteristics, the Simpson index was calculated for each season (winter, spring, summer) just considering the experiments carried out in Barcelona (WIBCN, SPBCN and SUBCN), and for each location and treatment considering the experiments carried out in summer (SUBCN, SUBLA and SUOFF) (Table 5.1). We also calculated the Simpson index for the experiments carried out in Barcelona and Blanes in spring and summer (SPBCN, SUBCN, SPBLA and SUBLA). In this case, we evaluated the differences between seasons (spring, summer), locations (Barcelona, Blanes), and treatments (C, A, S). To test whether Simpson varied significantly with respect the season, location and treatment, permutational multivariate analyses of variance (PERMANOVAs) based on Bray-Curtis distance (9999 permutations; $p < 0.05$) were computed. *p*-values were adjusted according to the false discovery rate in order to correct for multiple testing (Benjamini and Hochberg, 1995). These analyses were computed using the vegan package of the R software (v 3.2.4) (Oksanen et al., 2008).

$$D_2 = \frac{1}{\sum_{i=1}^{S} p_i^2} \qquad \text{Eq. 5.1}$$

, where pi is the proportion of species i, and S is the number of species.

An exploratory non-metric multidimensional scaling (NMDS) ordination and a heatmap based on Bray-Curtis distances were performed to visualize the distribution of the samples according to their phylogenetic composition. In the NMDS, each point represents one sample, including replicates (N = 58). For the heatmap, OTUs were grouped under taxonomic bacterial classes, and classes with a frequency lower than 10 reads in total were removed from the matrix. As the 10 most abundant classes accounted for 84% of the total OTUs and 99.65% of the total abundance, we only considered these 10 groups for the analyses performed at class scale. Relative abundances of the samples taken at TM and TF in WIBCN, SPBCN, and SPBLA were averaged (N = 4) for visualizing purpose in the heatmap. The vegan (Oksanen et al., 2008) and gplots (Warnes et al., 2013) packages were used.

Differences in read counts for individual OTUs observed between aerosol types within experiments were further evaluated with the DESeq2 package (Love et al., 2014). *p*-adjusted values according to the false discovery rate were calculated from a negative binomial distribution and significant differences were considered when *p*-adjusted was below an alpha cutoff value of 0.1. Statistical differences were also tested at class level. Although significant differences in certain OTUs were found between the samples filtered by 0.8 µm and 150 µm in SUBCN, and between the incubation time (TM *vs* TF) in WIBCN, SPBCN, and SPBLA (data not shown), we pooled all the samples from the same aerosol type (C, A, S) together to assess phylogenetic differences between treatments within experiments. In the experiments were significant differences were found, either at OTU or class level, the percentage of the relative abundance (% Rel. abundance) accounted for the taxonomic groups in which significant changes were

determined, was calculated by summing their abundances and dividing it by the total relative abundance within an experiment. CCAs were then computed for each of these experiments, to figure out the factors that may explain the observed differences in the BCC. In CCA, the ordination of samples and OTUs in the 2-dimensional space is constrained by the selected variables (Legendre and Legendre, 1988). Only the OTUs in which significant differences in their relative abundance between treatments were found are represented. The biogeochemical variables selected were: NO_3^-, TIP, OC (either as total – TOC – or dissolved – DOC –, avoiding co-linearity with other environmental variables), chlorophyll *a* (Chl), heterotrophic bacterial abundance (HBA) and production (HBP) – measured as leucine incorporation rate. Data were previously log-transformed. The ggvegan package (Wickham, 2009) was used for this purpose.

5.3. Results

5.3.1 Aerosol composition and release of nutrients into the seawater

The main chemical components of Saharan dust (S) in the collected filters were silica (SiO_2), which accounted for 30% of TSP on average, and aluminum oxide (Al_2O_3; 10% of TSP), while their contribution to anthropogenic aerosols (A) was 12% and 4%, respectively (see Table S5.2 for a detailed overview of the chemical composition of the two aerosol constituents). Total organic carbon (TOC) was the main component of anthropogenic particles (19% of TSP), while it only accounted for 6% in Saharan dust. Table S5.3 in the supplementary information shows the concentration of nutrients before and after the amendment in each treatment for the six experiments, at TM, and at TF. Following the amendment, TOC increased by, on average, 3.73 and 1.28 µM inside the A and the S microcosms, respectively, whereas it decreased in the controls. N, too, was an important component of both types of aerosols, especially the anthropogenic.

The percentages of nitrate (NO_3^-) and ammonium (NH_4^+) were 6.74% and 1.16%, respectively, in the A, and 3.76% and 0.41% in the S. This resulted in an average increase of 1.09 µM of NO_3^- and 0.57 µM of NH_4^+ in the A containers, while in the S, NO_3^- and NH_4^+ increased by 0.21 and 0.12 µM, accordingly (Table S5.3). The contribution of P was lower (0.12% of TSP in A and 0.09% in S) and only a small increase of 0.021 µM in total inorganic phosphorous (TIP) in the A and 0.017 µM in the S microcosms was detected, similar to what was observed in the controls (0.016 µM). Consequently, P was almost always depleted towards the end of the experiments, whereas N and TOC usually remained at higher concentrations in the amended containers, especially in the A. Nonparametric Wilcoxon tests showed that NO_3^- was significantly higher in the amended microcosms than in the controls ($p < 0.001$), and in the A than in the S ($p < 0.001$). NH_4^+ was also higher in the A ($p < 0.01$) and the S ($p < 0.05$) than in the C, whereas TIP and TOC were significantly higher in the A than in the C ($p < 0.05$ and $p < 0.01$, respectively), and TOC was also higher in the A than in the S ($p < 0.05$).

5.3.2 Effect of the environmental variables on alpha-diversity

Samples from the different experiments contained on average 375 OTUs and 6121 sequence reads each, and in all cases more than 1000 sequences. When OTUs were grouped under taxonomic classes, the most abundant (> 50% of the abundance) group were the *Alphaproteobacteria* (Fig. 5.1 and Table S5.4), and the SAR11 clade was the most abundant OTU in all the samples. *Cyanobacteria* (mainly of the genus *Synechoccocus*) were also remarkably abundant, being the second most important contributor in the samples of Blanes and offshore. *Gammaproteobacteria* and *Flavobacteria* were, on the contrary, more frequent in Barcelona, accounting for more

than 17% of the abundance in some of these samples. The contribution of *Deltaproteobacteria* reached more than 10% of the abundance in the experiment carried out in Barcelona during summer, but it was less frequent in the samples from the other experiments. The contribution attributed to the remaining groups (*Betaproteobacteria*, *Cytophaga*, *Sphingobacteria*, *Acidimicrobia* and *Opitutae)* was always less than 3% (Fig. 5.1).

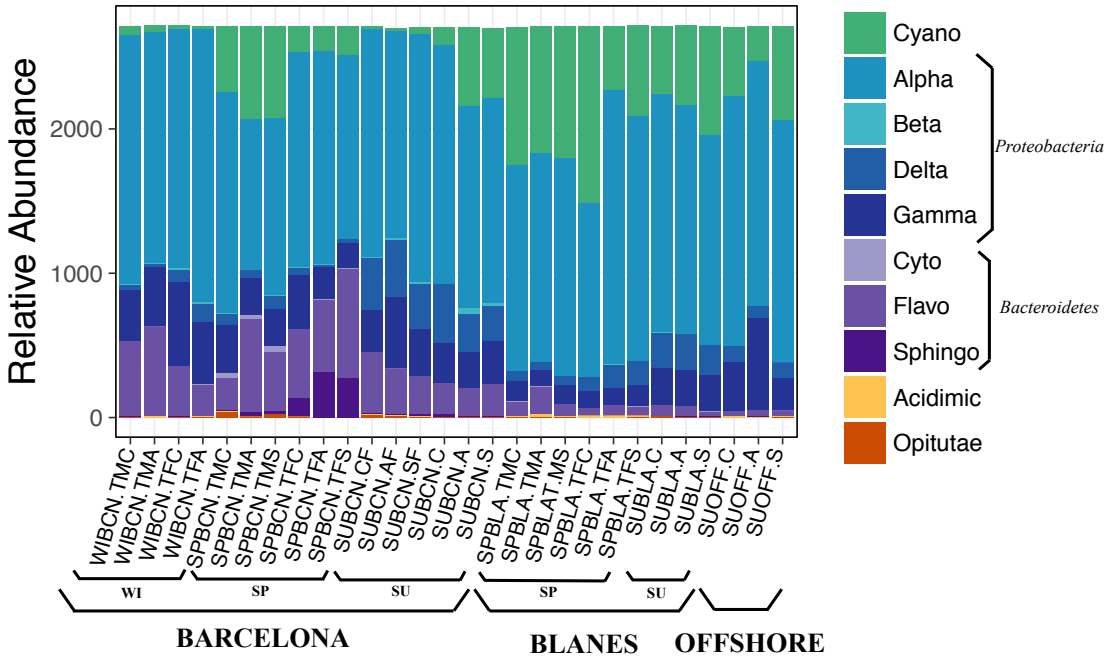


Figure 5.1. Abundance of the 10 most abundant marine groups in the samples, presented as the average from the two duplicate microcosms ($N = 2$). Abbreviations are as follow: Cyano = *Cyanobacteria;* Alpha = *Alphaproteobacteria*; Beta = *Betaproteobacteria*; Delta = *Deltaproteobacteria*; Gamma = *Gammaproteobacteria;* Cyto = *Cytophaga*; Flavo= *Flavobacteria*; Sphingo= *Sphingobacteria*; Acidimic = *Acidimicrobia*.

The Simpson diversity index was significantly higher in spring than in winter (p = 0.0029) and summer (p = 0.0019), and in SU than in WI (p = 0.0164) (Fig. 5.2a). In summer, diversity was significantly higher in BCN than in BLA (p = 0.0018) and OFF (p = 0.0025), and significantly higher in BLA than OFF (p = 0.0219) (Fig. 5.2b). Regarding the treatment, although diversity was slightly higher in the Saharan dust

samples (Fig. 5.2c), non-significant differences were found between treatments (p = 0.826). Results for the experiments carried out in Barcelona and Blanes in spring and summer (SPBCN, SUBCN, SPBLA and SUBLA) (see Section 5.2.4) gave similar results, being diversity significantly higher in Barcelona than in Blanes, and in spring than in summer (Figures not shown). When the Simpson index was calculated within experiments, in SUBCN, marginal significantly higher diversity was found in the A samples than in the other treatments (p = 0.0566 with respect the C, and p = 0.0888 with respect the S). Instead, filtration did not yield significant differences in diversity. Regarding the sampling time after aerosol amendment (TM with respect TF), significant differences in diversity were found in all the experiments where samples at TM were collected (p = 0.002 in WIBC, 0.0014 in SPBL, and 0.0283 in SPBC).

5.2.3 Effect of the environmental variables on beta-diversity

Exploratory non-metric multidimensional scaling (NMDS) ordination showed that samples from the same experiment clustered together (Fig. S5.1), revealing that location and seasonality were the main factors explaining the community composition of bacterioplankton in our samples. On the other hand, a heatmap considering the 10 most abundant marine groups showed that samples with the same treatment but from different experiments sometimes clustered closer than samples of the same experiment with different treatments (Fig. 5.3).

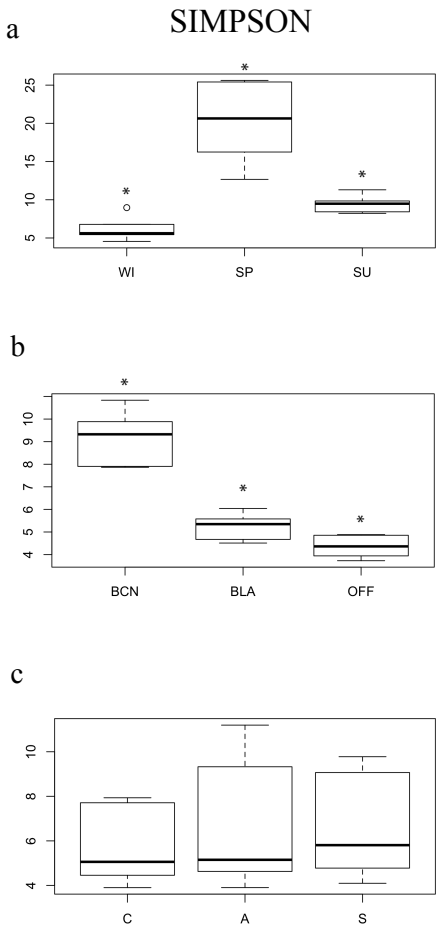


Figure 5.2. Box-plot representation of Simpson index for the different seasons in Barcelona (*N* = 17) (a); the different locations in summer (*N* = 18) (b), and the aerosol types in summer (*N* = 18) (c). The box indicates median and quartile values and the whiskers indicate the range (minima and maxima).

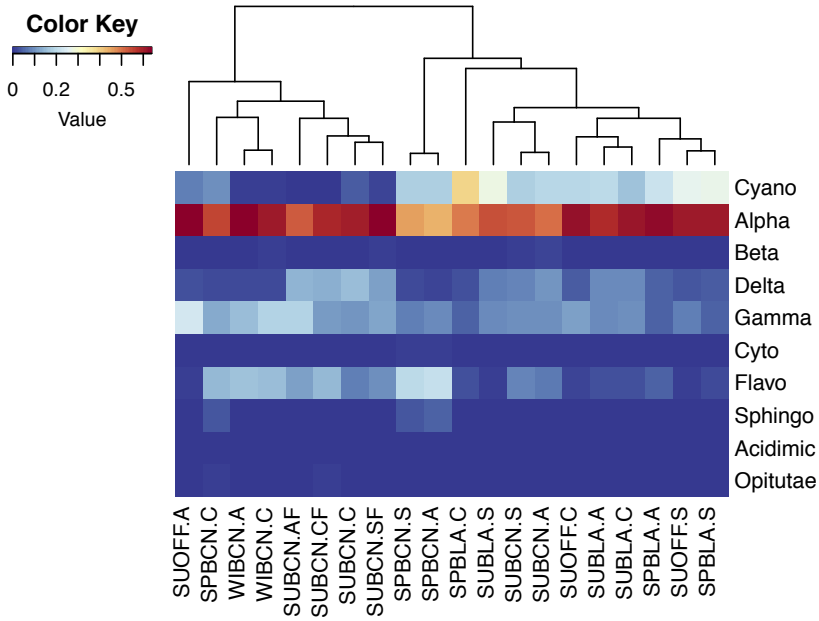


Figure 5.3. Heatmap showing the relative abundance of the 10 most abundant phylogenetic groups across the samples. Dendrogram distances are derived from pairwise Bray–Curtis distances. The names of the samples are composed by the name of the season (WI, SP, SU) plus the name of the location (BCN, BLA, OFF), followed by the aerosol type (C, A, S). The "F" indicates that samples were filtered by 0.8 µm (in SUBCN). Samples show average values from duplicate microcosms (C, A or S; *N = 2*). The names of the samples and abbreviations are as in Fig. 5.1, but in this case, samples taken at an intermediate time (TM) and at the end of the experiment (TF) in the experiments of WIBCN, SPBCN, and SPBLA, were averaged for visualization purpose.

5.2.4 Effect of aerosols at OTU and class levels

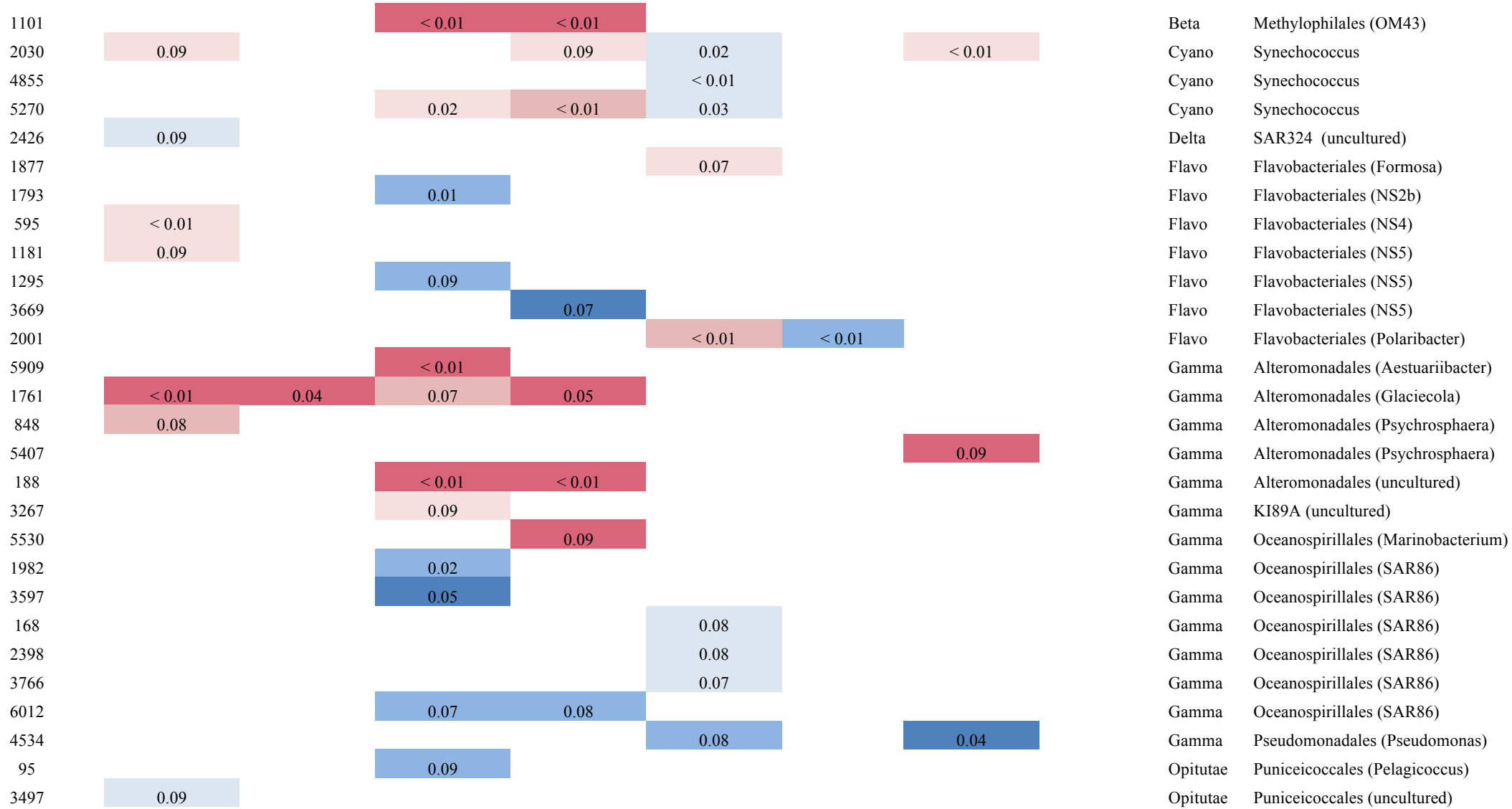
When the samples within a season and location were analyzed separately (i.e. considering a given experiment), statistical analyses revealed that the abundance of some bacterial taxa and groups varied significantly with the aerosol treatment (C, A, S). Significant differences were found within the experiments of Barcelona and Blanes in spring and summer (SPBCN, SUBCN, SPBLA and SUBLA; Fig. 5.4). In WIBCN and SUOFF, no significant changes in the abundance of any of the OTUs or marine groups were detected with the aerosols.

In Barcelona, anthropogenic aerosols produced significant changes in the phylogenetic composition of 26 OTUs in spring, which accounted for 48% of the bacterial relative abundance in this experiment (Fig. 5.4). From those 26, 21 were significantly more stimulated with the anthropogenic particles (mainly belonging to the *Alphaproteobacteria* class), and 5 were significantly less abundant, two of them belonging to the SAR11 clade. Saharan dust produced minor modifications in spring, with two OTUs significantly more stimulated in these samples than in the controls. In summer, both types of atmospheric particles yielded major changes in the BCC, 13 OTUs being significantly more abundant in the anthropogenic treatment and 14 in the Saharan than in the controls, whereas 7 OTUs where less abundant with the

anthropogenic particles and 3 with the Saharan dust. Furthermore, at class level, *Betaproteobacteria* and *Cyanobacteria* were significantly stimulated with both aerosols.

In Blanes, in spring, significant changes in OTU abundances were observed between the anthropogenic aerosols and the other treatments (C, S), but no changes were observed between the Saharan-amended samples and the controls (Fig. 5.4). OTUs belonging to *Alphaproteobacteria* and *Flavobacteria* were positively stimulated with the anthropogenic particles with respect to the controls and the Saharan dust, whereas three OTUs belonging to *Synechococcus* and four to *Gammaproteobacteria* were significantly less abundant in the A samples than in the controls. Again, in Blanes, the major alteration of the BCC in terms of relative abundance occurred in this experiment with the A particles, where the OTUs that significantly changed accounted for 32% of the community relative abundance. In summer, on the other hand, Saharan dust yielded changes at class scale that accounted for a shift in the relative abundance of the community of 10% at OTU level and 23% at class level (Fig. 5.4). *Cyanobacteria* (at class level, and one OTU of *Synechococcus* in particular), showed a significant increase in the Saharan containers than in the controls, whereas *Flavobacteria* and *Cytophaga* were significantly more abundant in both the C and the A treatments than in the S.

OTU (# Class)	SPBCN.A (C)	SPBCN.S (C)	SUBCN.A (C)	SUBCN.S (C)	SPBLA.A (C)	SPBLA.S (A)	SUBLA.S (C)	SUBLA.S (A)	Class	Order (Family/Genus)
3530					0.07				Alpha	OCS116 (uncultured)
5115				0.09					Alpha	Rhizobiales (Devosia)
1059	0.04								Alpha	Rhizobiales (Rhodobium)
2282	0.08								Alpha	Rhizobiales (Rhodobium)
4350	< 0.01								Alpha	Rhizobiales (Rhodobium)
3314	0.09								Alpha	Rhodobacterales (Ascidiaceihabitans)
179	< 0.01								Alpha	Rhodobacterales (Rhodobacteraceae)
485	0.09								Alpha	Rhodobacterales (Rhodobacteraceae)
2243	< 0.01	< 0.01							Alpha	Rhodobacterales (Rhodobacteraceae)
2763			< 0.01	< 0.01					Alpha	Rhodobacterales (Rhodobacteraceae)
5911	< 0.01								Alpha	Rhodobacterales (Rhodobacteraceae)
4058			< 0.01						Alpha	Rhodobacterales (Celeribacter)
590			0.01	0.09					Alpha	Rhodobacterales (Marivita)
1415	< 0.01								Alpha	Rhodobacterales (Nereida)
2730	< 0.01								Alpha	Rhodobacterales (Nereida)
4741	0.05								Alpha	Rhodobacterales (Octadecabacter)
5819	< 0.01								Alpha	Rhodobacterales (Planktomarina)
3790	< 0.01		0.02	< 0.01					Alpha	Rhodobacterales (Pseudophaeobacter)
244			< 0.01	< 0.01					Alpha	Rhodobacterales (Roseibacterium)
4281	0.09								Alpha	Rhodobacterales (Thalassobius)
2238	0.05								Alpha	Rhodospirillales (Defluviicoccus)
1710			< 0.01	< 0.01	0.09				Alpha	Rickettsiales (uncultured)
3151			< 0.01	0.02	0.02	0.09			Alpha	Rickettsiales (uncultured)
3437	< 0.01								Alpha	SAR11 (uncultured)
4357	0.09								Alpha	SAR11 (uncultured)
4417			0.09	< 0.01					Beta	Hydrogenophilales (uncultured)


1101 < 0.01 < 0.01 Beta Methylophilales (OM43)
2030 0.09 0.09 0.02 < 0.01 Cyano Synechococcus
4855 < 0.01 Cyano Synechococcus
5270 0.02 < 0.01 0.03 Cyano Synechococcus
2426 0.09 Delta SAR324 (uncultured)
1877 0.07 Flavo Flavobacteriales (Formosa)
1793 0.01 Flavo Flavobacteriales (NS2b)
595 < 0.01 Flavo Flavobacteriales (NS4)
1181 0.09 Flavo Flavobacteriales (NS5)
1295 0.09 Flavo Flavobacteriales (NS5)
3669 0.07 Flavo Flavobacteriales (NS5)
2001 < 0.01 < 0.01 Flavo Flavobacteriales (Polaribacter)
5909 < 0.01 Gamma Alteromonadales (Aestuariibacter)
1761 < 0.01 0.04 0.07 0.05 Gamma Alteromonadales (Glaciecola)
848 0.08 Gamma Alteromonadales (Psychrosphaera)
5407 0.09 Gamma Alteromonadales (Psychrosphaera)
188 < 0.01 < 0.01 Gamma Alteromonadales (uncultured)
3267 0.09 Gamma KI89A (uncultured)
5530 0.09 Gamma Oceanospirillales (Marinobacterium)
1982 0.02 Gamma Oceanospirillales (SAR86)
3597 0.05 Gamma Oceanospirillales (SAR86)
168 0.08 Gamma Oceanospirillales (SAR86)
2398 0.08 Gamma Oceanospirillales (SAR86)
3766 0.07 Gamma Oceanospirillales (SAR86)
6012 0.07 0.08 Gamma Oceanospirillales (SAR86)
4534 0.08 0.04 Gamma Pseudomonadales (Pseudomonas)
95 0.09 Opitutae Puniceicoccales (Pelagicoccus)
3497 0.09 Opitutae Puniceicoccales (uncultured)

3738	0.02								Sphingo	Sphingobacteriales (uncultured)
6049	0.01								Sphingo	Sphingobacteriales (uncultured)
% Rel. abundance	**48**	**1**	**10**	**9**	**32**	**2**	**10**			
#10			< 0.01	< 0.01					Beta	
#2			0.04	< 0.01			0.07		Cyano	
#9							< 0.01	0.02	Cyto	
#4						0.09	< 0.01	< 0.01	Flavo	
% Rel. abundance			**7**	**7**		**3**	**23**	**4**		

Legend (log_2-Fold Change)

From -6 to -4
From -4 to -2
From -2 to 0
From 0 to 2
From 2 to 4
From 4 to 6
From 6 to 8

Figure 5.4. Relative change in the abundance of certain OTUs or marine class (# Class) observed between treatments (C, A, S) within experiments, as revealed by DESeq2 analyses. The colored legend represents the $\log_2$-fold change in the relative abundance of a particular OTU in a particular sample with respect another (specified in parenthesis). The chromatic colored legend indicates the changes in ranges from -6 to 8. *p*-values are indicated for each particular OTU in a given sample. % Rel. abundance represents the change in the relative abundance of the bacterial community yielded by a given aerosol treatment, either as the sum of the changes produced in individual OTUs or at class level.

5.4. Discussion

5.4.1 Effect of the environmental variables on bacterial diversity

The main objective of this study was to evaluate the effect of different types of atmospheric particles on the bacterial community composition in surface waters of the NW Mediterranean, at different times of the year, and in locations with different anthropogenic footprint. As expected, bacterial diversity was mainly determined by the season and the location. These results confirm previous studies where seasonality was suggested to be a predominant factor modulating the structure of bacterioplankton in the NW Mediterranean (Alonso-Sáez et al., 2007; Pinhassi et al., 2006; Schauer et al., 2003). The location has been observed to shape the BCC in coastal waters of the NW Mediterranean too (Nogales et al., 2007; Schauer et al., 2000), although other studies showed only slight changes among locations within the western Mediterranean (Acinas et al., 1997).

According to general ecological theory, more eutrophic systems are expected to be less diverse (Frontier, 1985). However, we found the highest diversity (measured as inverse Simpson indices) in Barcelona (urban coastal location), the location with the low degree of oligotrophy, due to higher and disparate sources of nutrients and organic compounds in coastal areas, especially those surrounding urban locations (Nogales et al., 2007; Romero et al., 2014; Schauer et al., 2000). Schauer et al. (2000) determined similar

Simpson indices in different coastal sites along the Catalan coast, but higher in the Barcelona harbor, the most anthropogenically-altered site. Similarly, Nogales et al. (2007) found a higher diversity in a highly impacted marina from a coastal area in the Mallorca Island (Spain) than in a more isolated bay. In our case, the differences observed between locations may also be attributed to the aerosols, as they yielded major changes in the bacterial community composition in Barcelona and Blanes, but not in Mallorca. Diversity was also larger in spring than in winter and summer. Alonso-Sáez et al. (2007), in contrast, found a lower diversity in spring in BCC than during the rest of the year in Blanes. Again, our results may be attributed to the major changes observed in the Barcelona and Blanes samples during spring, where aerosols (mainly from anthropogenic origin) drove shifts in the prokaryotic community that accounted for 48% and 32% of the relative abundance within these experiments, respectively (Fig. 5.4). It must also be considered that a "real" Saharan dust event occurred in the NE of Spain the week previous to the Blanes experiment in spring (April, 1st – 3rd, 2014). Saharan deposition could have release new nutrients into the seawater that might be limiting the growth of certain species, thus increasing bacterioplankton diversity with respect usual conditions at that time of the year.

On the other hand, the Simpson index was higher in the Saharan-dust samples, but the variability was quite high (Fig 5.2c), which prevented us from finding a clear pattern in diversity with respect the aerosol treatment. In previous experiments where Saharan dust was added to planktonic communities, no significant differences in bacterial diversity have been found either (Pulido-Villena et al., 2014; Reche et al., 2009). While, experiments in which the effect of mixed dry particles or rainwater on the BCC was assessed did not quantified the net change in diversity (Guo et al., 2016; Teira et al.,

2013). In Barcelona, in the summer experiment, a higher diversity was found with the anthropogenic samples, but overall, our results suggest that atmospheric particles of both sources have the potential to change the composition of the bacterial community by releasing different types of compounds that will favor some species while preclude the growth of others, thus not yielding an increase either a decrease in the net diversity of the community. The compounds released by the aerosols will depend on their area source, the chemical processes suffered in the atmosphere until deposition, as well as on the biogeochemical conditions present in the seawater (Longo et al., 2014; Marín et al., 2017; Moreno et al., 2006).

5.4.2 Effect of aerosols on the bacterial community composition

When the effect of aerosols was evaluated for each experiment, atmospheric particles produced significant changes in the abundance of certain OTUs and marine groups. To investigate the reasons associated to the observed changes, we carried out canonical correspondence analyses (CCA) representing the OTUs that significantly changed within the experiments of Barcelona and Blanes in spring and summer, and the main biogeochemical variables studied (Fig. 5.5).

In Barcelona, anthropogenic particles yielded major changes in spring. A significantly higher abundance was determined in OTUs belonging to *Alphaproteobacteria* (mainly from the orders *Rhizobiales* and *Rhodobacterales*), *Gammaproteobacteria* (*Alteromonadales*), *Flavobacteriales* and *Sphingobacteriales* (both belonging to the phylum *Bacteroidetes*). *Rhodobacterales* and *Gammaproteobacteria* are groups generally associated with algal blooms (Lebaron et al., 1999; Pinhassi et al., 2004), and *Flavobacteria* and *Sphingobacteria* have been observed to benefit from organic sources

of N and P, respectively (Suzuki et al., 2001). Hence, their stimulation in spring in the A microcosms might be attributed to the release of organic compounds from the aerosols or from phytoplankton cells, as a peak in Chl at TM in the amended containers and a posterior decay at TF was observed (in Fig. 5.5a, TM-A and TM-S samples appear close to Chl). The CCA (p = 0.004, 88% of the variance explained by the selected variables) shows that several OTUs of *Rhodobacterales* (4741, 4281, 5911) are closely related to the HBP in this experiment, suggesting that *Rhodobacterales* are the main responsible of the high increase in HBP observed in the A containers at the end of this experiment, and related with high TOC concentrations. In contrast, SAR11, that are the most abundant bacterial group in the ocean (Alonso-Sáez et al., 2007; Giovannoni and Rappé, 2000), significantly decreased with the anthropogenic particles in spring. A decrease in SAR11 upon Saharan dust addition has previously been reported in two different studies carried out in the Atlantic Ocean (Hill et al., 2010; Marañón et al., 2010), and also with mixed aerosols in the eastern Mediterranean (Guo et al., 2016). Compared to other phylogenetic groups, SAR11 did not respond to rainwater additions either in the NW coast of Spain (Teira et al., 2013). Nogales et al. (2007) observed that the abundance of active SAR11 decreased in the most nutrient-enriched areas in the island of Mallorca. Our results also show that the abundance of SAR11 OTUs (3437 and 4357; Fig. 5.5a) seems to be anti-correlated with increasing nutrient concentration (especially nitrate).

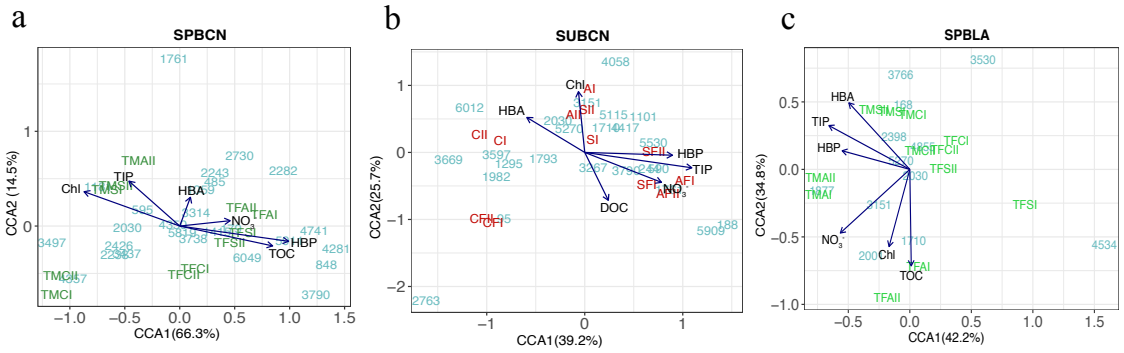


Figure 5.5. Bi-plot visualization of the canonical correspondence analyses (CCAs) carried out with the samples of Barcelona in spring (a), and summer (b), and in Blanes during spring (c). The experimental samples are represented in dark green (SPBCN), red (SUBCN), and light green (SPBLA), and the OTUs in cyan. In SPBCN (N = 11) and SPBLA (N = 12), samples were collected at TM and TF. In SUBCN samples filtered by 150 µm and 0.8 µm were collected at TF (N = 12).

Rhodobacterales were also among the main contributors to HBP in summer, together with *Gammaproteobacteria* (Figs. 5.4 and 5.5b; 81% of the variance in the CCA was explained by the selected variables, p = 0.002). This is consistent with previous studies were *Alphaproteobacteria* have been shown to be the dominant contributors to bacteria production in seawater (Cottrell and Kirchman, 2003; Zubkov et al., 2001), what has been attributed to the presence of low–molecular weight dissolved organic matter (Cottrell and Kirchman, 2003). These compounds may have been released from the phytoplankton during spring but this is unlikely in summer, as in this experiment the initial concentration of Chl was quite low (0.44 µg l^{-1}) and it only slowly increased towards the end of the experiment in the amended microcosms (see Fig. 3.3a; Chapter 3), when the samples for BCC were taken. Furthermore, a large and quick stimulation in HBP was observed in the samples filtered by 0.8 µm (see Fig. 4.4; Chapter 4), where only low chlorophyll corresponding to *Synechococcus* could be expected. Hence, in summer, the observed increase in *Alpha-* (*Rhodobacterales and Rickettsiales*), *Beta-, Gammaproteobacteria* (*Alteromonadales*), and *Cyanobacteria* with both types of

aerosols must be attributed mainly to the direct assimilation of inorganic nutrients (NO_3, TIP) released by aerosols. This is in agreement with the idea that smaller cells can outcompete phytoplankton in more oligotrophic conditions (Marañón et al., 2010; Raven, 1998). Although OC was significantly higher in the A containers, the dissolved fraction did not seem to affect the bacterial assemblages during summer (Fig. 5.5b), probably because the OC concentration in the seawater at the beginning of this experiment largely exceeded that of total inorganic N (TIN) and TIP, with a ratio of TOC:TIN:TIP of 1768:46:1.

In Blanes, Saharan and anthropogenic particles produced disparate responses in the BCC both at OTU and class levels. In spring, a couple of OTUs of *Rickettsiales* and other two of *Flavobacteriales* were significantly more abundant in the A samples, and related with higher contents in TOC, Chl, and NO_3^- (Figs. 5.4 and 5.5c, CCA significance was p = 0.002, and 92% of the variance is explained by the selected variables), which suggest that these heterotrophic bacteria have preference for compounds richer in N and OC, as previously reported (Suzuki et al., 2001). *Flavobacteria* were also more abundant in the A than in the S samples at class scale, which may be related with both, the major stimulation of Chl in the A containers, and the major release of organic nitrogen from anthropogenic particles, as also suggested by higher values of the enzyme leu-aminopeptidase in the anthropogenic samples (see Chapter 4 for more detailed information about enzymatic activity during these experiments). Indeed, the organic fraction of N has been determined to account for a large percentage of total N in anthropogenic aerosols (Cornell et al., 2003; Markaki et al., 2010). In summer, Saharan dust yielded changes in bacterial relative abundance that accounted for 10% at OTU level and 23% at class level. However, as only 3 OTUs

showed significant differences between treatments, the CCA analysis performed at OTU level was non-significant in this case. Saharan dust significantly stimulated the growth of *Cyanobacteria* in this experiment, while two groups of *Bacteroidetes* (*Cytophaga* and *Flavobacteria*) were less abundant in the Saharan than in the other treatments. *Cyanobacteria* may have outcompeted larger phytoplankton cells for inorganic nutrients in the Saharan treatment, whereas nanoeukaryotes and diatoms grew more in the anthropogenic samples (Sdena Nunes personal communication).

Putting all these results together and including the incubation time as continuous variable, the larger increases in the total prokaryotic abundance and production were observed in Barcelona during spring, and related with higher content in organic carbon in the amended samples (Fig. 5.6, $p = 0.001$, 53% of the total variance is explained by the selected variables). At the intermediate time (TM), samples were more correlated with high nitrate and phosphate contents, but these nutrients were consumed at the end of the experiment (Table S5.2). In contrast, nitrate concentration remained higher at the end of the summer experiment in Blanes, and to a lesser extent during the spring experiment as well. The summer experiment of Barcelona instead was characterized by lower nutrient concentrations, especially of TIP, at the end of the experiment, as it was quickly consumed due to starvation P conditions at the beginning of this experiment (Table S5.2, see also Chapter 3 for more detailed information of nutrient conditions in the experiments of Barcelona). A concomitant lower stimulation of the biological variables (including Chl, not shown in Fig.5.6 as it was highly correlated with TIP) was also observed in this experiment compared to those carried out in spring and in Blanes during summer.

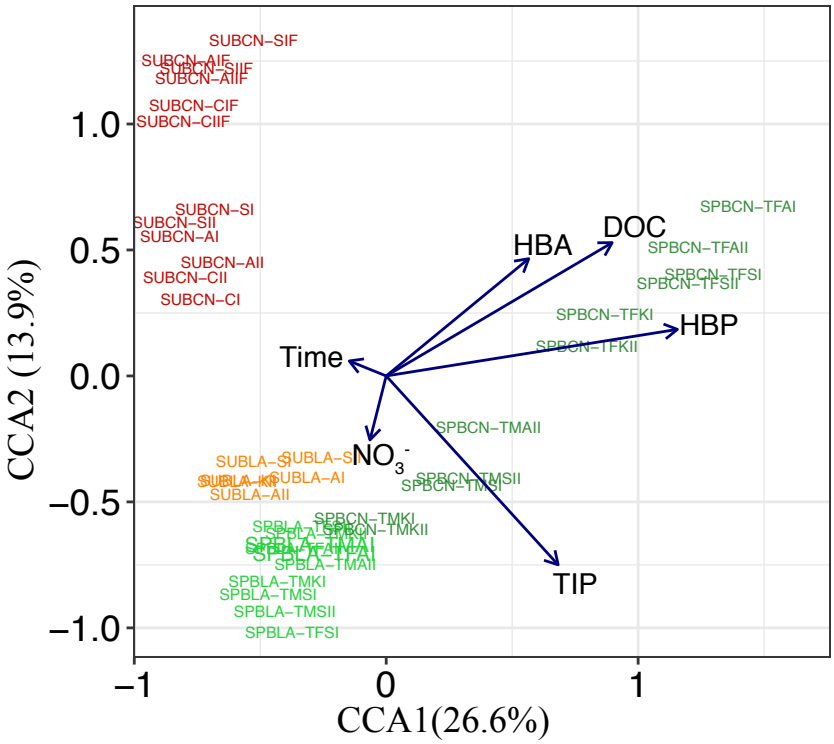


Figure 5.6. CCA representing the samples of all the experiments in which significant differences for certain OTUs and bacterial groups were found between treatments (SPBCN, SUBCN, SPBLA, SUBLA). The experimental samples are represented in dark green (SPBCN), red (SUBCN), light green (SPBLA) and orange (SUBLA). OTUs are not presented for visualizing purpose.

On the other hand, no significant changes in the abundance of any of the OTUs or marine groups were detected in Barcelona during winter or in the experiment carried out offshore. Although BCC is certainly very similar within the winter samples, some differences between treatments are evident in the offshore experiment (Figs. 5.1 and 5.3). It must be considered that the incubation time in this experiment was the shortest (2 days since the aerosol additions, Table 5.1), and fewer samples than in other experiments were collected (N = 6, Table 1), which may have prevented us from detecting significant differences between aerosol types. Similarly, Laghdass *et al.* (2011) did not find noticeable differences between samples amended with Saharan dust

and the controls during the DUNE 1 experiment 8 days after the seeding, whereas Pulido-Villena *et al.* (2014) did observe significant changes in the abundance of a few OTUs in a similar mesocosm experiment sampling at a higher temporal resolution (DUNE-R).

Conclusions

This study provides new insights into the effect of atmospheric deposition of different origin on bacterial assemblages at different times of the year in the NW Mediterranean. We found that certain taxa and marine groups of relevant abundance in the whole community underwent significant changes with aerosols during spring and summer, although the overall result is clouded by other more relevant factors determining the structure of the bacterial community composition, such as season and geographical location. Other factors, such as the presence of competitors and predators, and the incubation time after amendment, are important to properly evaluate the effect of aerosols on the bacterial community. It can be said therefore that aerosols from either mineral and anthropogenic origin have the potential to change the phylogenetic composition of marine bacteria at certain times of the year and locations in oligotrophic areas as the Mediterranean. In our experiments, anthropogenic particles favored more the growth of heterotrophic bacteria (mainly from the groups *Alphaproteobacteria* and *Bacteroidetes*) than Saharan dust, due to either a direct effect of fertilization by providing inorganic P, N, and organic compounds, or as an indirect effect following the stimulation of phytoplankton. The effect of anthropogenic aerosols was overall higher in spring, probably associated with the lower concentration of organic carbon compared with inorganic nutrients at this time of the year compared to summer. Saharan dust, instead, produced a major stimulation of *Cyanobacteria* in summer, and mainly

associated to the release of inorganic N. These observed differences might ultimately yield changes in the C cycle, as *Cyanobacteria* take up CO_2 to carry the photosynthesis, and heterotrophic bacteria mineralize the organic matter, releasing part of the CO_2 back to the atmosphere.

Acknowledgments

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Supplementary Material

Supplementary Methods S1. In-detail description of aerosol collection, classification, and inoculum preparation.

Aerosol Collection

Aerosols – in the form of total suspended particles (TSP) – were sampled in Barcelona (BCN) at the Institut de Ciencies del Mar (41.39°N, 2.20°E) and in Blanes (BLA) at the Centre d'Estudis Avancats (41.68°N, 2.80°E). Aerosol collection, with regard to time points, was carried out based on forecast presence/absence of Saharan dust intrusions to the Iberian Peninsula (provided by www.calima.ws; see further down): Saharan (S) particles were collected on June, 7th (2013), August, 5th (2013), February, 18 th (2014), and April, 1st – 3rd (2014) in Barcelona; and on February, 18 – 20th (2014) in Blanes (the same filter was employed for the two experiments carried out in Blanes). Anthropogenic (A) particles were collected on April, 10th (2013), July, 4th (2013), August, 12th (2013), December, 17th (2013), January, 15th, 17th and 27st (2014), February, 17th (2014), and July, 22 – 23th and 28 – 29th (2014) in Barcelona; and on January, 21 – 23rd (2014), February, 11 – 13rd (2014), March, 18 – 20th and 25 – 24th, May, 6 – 9th, and June, 2 – 5th in Blanes (note that particles were collected during more days in Blanes to reach similar concentrations to that in Barcelona). Once the gravimetric determination of the particulate matter trapped in the filters was performed, filters were cut into two equal sections. Half of the filter was kept at 4 ºC and employed to characterize the chemical composition of the particles, and the other half was frozen at −20 ºC until as inoculum in the amendment experiments.

Aerosol Classification

Classification of aerosols as Saharan dust (S) or anthropogenically-derived (A) was based on (1) the predicted presence (S) or absence (A) of Saharan dust intrusions to the Iberian Peninsula and (2) subsequent verification of the collected aerosol filters via chemical analysis.

The former integrated four approaches: 1) interpretation of daily meteorological conditions and daily air mass trajectories calculated at noon for a given day and for five days ago (at 750, 1500, and 2500 m above sea level), using the model HYSPLIT (Hybrid Single-Particles Lagrangian Integrated Trajectories; http://ready.arl.noaa.gov/HYSPLIT.php); 2) maps of the Ozone Monitoring Instrument Aerosol Index (ftp://toms.gsfc.nasa.gov/pub/omi/images/aerosol/) as well as daily satellite images from NASA (http://oceancolor.gsfc.nasa.gov/SeaWiFS/HTML/dust.html); 3) results from simulations using the models SKIRON (University of Athens; Athens, Greece), DREAM (Barcelona Supercomputing Centre; Barcelona, Spain), and NAAPS (US Naval Research Laboratory at Monterrey; Monterrey, USA); and 4) application of the model HIRLAM-AEMET with regard to wind trajectories (http://www.aemet.es/es/eltiempo/prediccion/modelosnumericos/hirlam005). All four approaches were integrated to provide a forecast of the presence or absence of Saharan dust intrusions to the Iberian Peninsula; hence, allowing sampling of aerosols at time points predicted to be of mineral (S) or rather anthropogenic (A) origin.

Chemical analysis of the collected filters was subsequently carried out to verify the aerosols' origin. The analyses were done following the methodologies found elsewhere

(Moreno et al., 2006; Querol et al., 2001). For this purpose, a number of ratios among different chemical elements were calculated (P:Al, Fe:Al, Zn:Al, Pb:Al, Cd:Al, Si:Ca, Si:Fe, Al:Ca, Al:Fe, Ti:Ca and Ti:Fe). Threshold ratios of 0.012, 0.63, $1.01.10^{-3}$, $3.41.10^{-4}$, and $1.71.10^{-6}$ exist for P:Al, Fe:Al, Zn:Al, Pb:Al, and Cd:Al, respectively (Guieu et al., 2010, and references therein): observed ratios below and above these threshold values would classify the samples as of S and A, respectively. Moreover, high ratios of Si:Ca, Si:Fe, Al:Ca, Al:Fe, Ti:Ca and Ti:Fe would be attributed to Saharan dust (Nava et al., 2012). To further distinguish Saharan dust from anthropogenically-derived aerosols, the enrichment factor (EF) was calculated for trace metals as described in Migon et al. (2001):

$$EF_M = \frac{(\frac{M}{Al})_{AE}}{(\frac{M}{Al})_{CR}} \qquad \text{Eq. S5.1}$$

, where M is the concentration of the metal recorded in the aerosol (AE) or in the continental crust (CR). The concentrations of M and Al in CR are the ones reported by Wedepohl (1995). Ratios close to 1 are typical of crustal origin aerosols, while values above 10 normally belong to anthropogenically-derived aerosols (Migon et al., 2001).

Aerosol inoculum preparation

Particles were extracted from the filters into 250 ml of artificial seawater (NaCl EMSURE, Grade ACS, Merck; Darmstadt, Germany) by sonication for 20 min (7 kHz) in a Bandelin SONOREX Digital 10 P Ultrasonic bath (Sigma-Aldrich, Merck; Darmstadt, Germany). This solution was used to inoculate the microcosm stimulation experiments.

Supplementary Methods S2. In-detail description of PCR amplification and pyrosequencing

The bacterial hypervariable regions V1, V2, and V3 of the 16S rRNA gene were PCR amplified, using a forward and a reverse fusion primer 28F (5'-GAGTTTGATCNTGGCTCAG-3') and 519R (5'-GTNTTACNGCGGCKGCTG-3') (Handl et al., 2011), respectively. The primers were modified in advance according to the final configuration: AdaptorA-MID-28F and biotin-AdaptorB-519R (AdaptorA and B are 454 Life Sciences adaptor sequences; Branford, CT, USA). Multiplex identifiers (MID) were 8-10 nucleotides long and sample specific. Amplifications were performed in a 25-µl reaction volume made up of 1µl of each primer (5 µM), 1µl of template, and 22 µl of the Qiagen HotStar Taq master mix (Qiagen Inc; Valencia, CA, USA). Reactions were performed on ABI Veriti thermocyclers (Applied Biosytems; Carlsbad, CA, USA) according to the following thermal profile: 95 °C for 5 min, 35 cycles of 94 °C for 30 sec, 54 °C for 40 sec, and 72 °C for 1 min, and finalized by one cycle at 72 °C for 10 min. PCR amplicons were then pooled equimolarly and cleaned using the Agencourt AMPure XP purification kit (BeckmanCoulter Inc.; Brea, CA, USA). The final, pooled amplicon was re-quantified and diluted accordingly, upon which it was used in emulsion PCR. Sequencing was performed on a 454 GS-FLX+ system (454 Life Sciences) at the Research and Testing Laboratory of Lubbock (http://rtlgenomics.com/; TX, USA).

Table S5.1. Results of the ANCOVA performed for each measured variable, using the type of control as categorical variable and the time as covariate. The factors were CONTROL, EXPERIMENT, and TIME (only results for CONTROL are shown). The number of samples (*N*), R^2 adjusted and the *p*-value are detailed for each of the variables. **p* <0.01; ***p* <0.0001; n.s.: non-significant. Abbreviations are as follow: TIP = total inorganic phosphorous; TOC = total organic carbon; DOC = dissolved organic carbon; Chl = chlorophyll *a*; HBA = heterotrophic bacterial abundance; HBP = heterotrophic bacterial production; Syn = *Synechococcus*; Proc = *Prochlorococcus*; Pico = picoeukaryotes; Nano = nanoeukaryotes.

	N	**R^2 adj**	***p*-value**	**CONTROL**
NO_3^-	114	0.668	**	n.s.
NO_2^-	114	0.494	**	n.s.
NH_4^-	114	0.201	**	n.s.
TIP	114	0.891	**	n.s.
SiO_4^{4-}	114	0.990	**	n.s.
TOC	64	0.823	**	n.s.
DOC	39	0.681	**	n.s.
Chl	96	0.666	**	n.s.
HBA	96	0.842	**	n.s.
HBP	64	0.269	**	n.s.
Syn	96	0.687	**	*(p = 0.0046)
Proc	96	0.243	**	n.s.
Pico	96	0.535	**	n.s.
Nano	96	0.684	**	n.s.

Table S5.2. Chemical composition (mean ± standard deviation) of the anthropogenic (A) and Saharan (S) filters used as inoculum for the amendment experiments. Results are expressed in µg m^{-3}, and as percentage of total suspended particles (%TSP). Abbreviations: TOC = total organic carbon, EC = elemental carbon.

	A		S	
	µg m^{-3}	% TSP	µg m^{-3}	% TSP
TSP	57 ± 45		138 ± 52	
SiO_2	10.34 ± 16.94	12.15 ± 10.72	42.82 ± 26.31	30.02 ± 11.10
Al_2O_3	3.45 ± 5.65	4.05 ± 3.57	14.26 ± 8.76	10.00 ± 3.70
TOC	7.46 ± 3.50	18.79 ± 12.44	8.24 ± 3.20	6.23 ± 1.64
EC	2.06 ± 1.72	4.49 ± 2.82	1.70 ± 0.78	1.38 ± 0.70
CO_3	3.80 ± 3.49	6.34 ± 3.38	9.43 ± 6.09	6.50 ± 2.29
NO_3^-	3.31 ± 2.43	6.74 ± 3.91	5.06 ± 2.84	3.76 ± 1.41
NH_4^+	0.52 ± 0.63	1.16 ± 1.03	0.55 ± 0.35	0.41 ± 0.17
P	0.06 ± 0.04	0.12 ± 0.07	0.13 ± 0.08	0.09 ± 0.03
Ca	2.53 ± 2.32	4.23 ± 2.25	6.30 ± 4.08	4.34 ± 1.53
Fe	1.52 ± 1.77	2.25 ± 1.25	4.67 ± 2.77	3.24 ± 0.99
K	0.95 ± 1.08	1.49 ± 0.65	2.64 ± 1.77	1.86 ± 0.69
Na	2.24 ± 2.09	3.95 ± 1.86	5.75 ± 4.70	3.82 ± 1.99
Mg	0.69 ± 0.80	1.02 ± 0.45	2.19 ± 1.58	1.50 ± 0.62
SO_4^{2-}	2.18 ± 1.44	4.56 ± 2.14	5.12 ± 2.63	3.97 ± 1.92
Cl^-	2.35 ± 2.74	3.99 ± 3.49	6.58 ± 6.20	3.94 ± 2.94

Table S5.3. Average concentration of NO_3^-, NH_4^+, total inorganic phosphorous (TIP), total organic carbon (TOC), chlorophyll *a* (Chl), heterotrophic bacterial abundance (HBA) and production (HBP) in the duplicate containers ($N = 2$) for each of the experiments. Samples were measured before the aerosols addition (TB), after the addition (TA), in the middle time were samples for BCC were taken (TM), and at the end of each experiments (TF). The difference between TA and TB was calculated for each of the nutrients and the average value for all the experiments is shown (TA - TB Average).

	NO_3^-					NH_4^+					TIP					TOC				
	TB	TA	TA-TB	TM	TF	TB	TA	TA-TB	TM	TF	TB	TA	TA-TB	TM	TF	TB	TA	TA-TB	TM	TF
WIBC.C	0.785	1.001	0.216	0.720	0.266	0.256	0.292	0.036	0.093	0.084	0.057	0.080	0.023	0.029	0.021	71.790	69.350	-2.440	71.235	79.145
WIBC.A	0.924	2.227	1.303	1.928	1.652	0.269	0.960	0.691	0.585	0.486	0.051	0.080	0.029	0.028	0.024	67.728	73.820	6.093	71.315	79.913
SPBC.C	1.906	1.338	-0.568	0.347	0.415	0.359	0.127	-0.232	0.100	0.120	0.178	0.202	0.024	0.149	0.097	88.350	94.380	6.030	97.920	108.100
SPBC.A	2.375	2.632	0.257	0.322	1.610	0.500	0.632	0.132	0.097	0.112	0.225	0.235	0.009	0.158	0.142	85.970	92.740	6.770	98.290	115.900
SPBC.S	2.435	1.277	-1.158	0.320	0.342	0.517	0.384	-0.134	0.092	0.084	0.229	0.226	-0.003	0.166	0.119	86.095	92.380	6.285	92.300	103.800
SUBC.CF	0.680	n.d.	-	-	0.703	0.197	n.d.	-	-	0.207	0.016	n.d.	-	-	0.014	96.900	n.d.	-	-	92.200
SUBC.AF	0.667	1.464	0.798	-	1.481	0.099	0.243	0.145	-	0.636	0.013	0.048	0.035	-	0.044	96.300	95.550	-0.750	-	121.066
SUBC.SF	0.446	1.058	0.612	-	0.951	0.132	0.383	0.251	-	1.096	0.014	0.054	0.040	-	0.033	90.000	99.800	9.800	-	86.355
SUBC.C	0.425	n.d.	-	-	0.148	0.201	n.d.	-	-	1.231	0.035	n.d.	-	-	0.017	96.500	n.d.	-	-	74.178
SUBC.A	0.776	1.694	0.918	-	0.761	0.124	0.292	0.169	-	0.172	0.028	0.053	0.025	-	0.025	93.150	95.600	2.450	-	89.365
SUBC.S	0.479	0.943	0.464	-	0.296	0.048	0.198	0.150	-	1.198	0.029	0.070	0.041	-	0.014	98.200	97.650	-0.550	-	100.895
SPBL.C	1.275	1.265	-0.010	1.205	0.458	0.015	0.056	0.041	0.126	0.094	0.140	0.148	0.008	0.151	0.151	69.545	68.640	-0.905	69.910	69.845
SPBL.A	1.385	3.805	2.420	3.975	4.145	0.023	1.975	1.952	1.569	0.530	0.145	0.163	0.018	0.167	0.123	68.530	74.765	6.235	76.755	79.580
SPBL.S	1.165	1.815	0.650	2.010	1.325	0.027	0.212	0.185	0.101	0.124	0.150	0.152	0.002	0.155	0.117	68.655	68.370	-0.285	66.845	77.590
SUBL.C	0.414	0.093	-0.321	-	0.055	0.067	0.159	0.093	-	0.069	0.053	0.061	0.008	-	0.028	81.270	79.345	-1.925	-	87.585
SUBL.A	0.337	1.390	1.053	-	1.148	0.024	0.665	0.642	-	0.119	0.058	0.052	-0.006	-	0.036	81.065	82.535	1.470	-	93.685
SUBL.S	0.417	0.636	0.219	-	0.518	0.106	0.231	0.125	-	0.083	0.075	0.071	-0.004	-	0.028	83.940	79.090	-4.850	-	93.235
SUMA.C	0.338	0.205	-0.134	-	0.136	0.159	0.165	0.006	-	0.209	0.041	0.059	0.018	-	0.080	81.690	80.528	-1.162	-	84.598
SUMA.A	0.338	1.000	0.662	-	0.848	0.159	0.318	0.159	-	0.630	0.041	0.067	0.026	-	0.038	81.690	83.130	1.440	-	79.715
SUMA.S	0.338	0.794	0.456	-	0.658	0.159	0.304	0.145	-	0.413	0.041	0.065	0.024	-	0.044	81.690	78.975	-2.715	-	81.900

TA-TB (Average)				
C	-0.163	-0.011	0.016	-0.080
A	1.089	0.573	0.021	3.725
S	0.207	0.120	0.017	1.281

Table S5.4. Taxonomic composition of the 10 most frequent marine groups determined in the sample-by-species matrix after normalize to 2717 reads. Relative abundances are the average from the two duplicate microcosms ($N = 2$) at a given time. The names of the samples and abbreviations are as in Fig. S1.

	Cyano	Alpha	Beta	Delta	Gamma	Cyto	Flavo	Sphingo	Acidimic	Opitutae
WIBCN.TMC	58	1731	6	30	354	1	526	1	4	3
WIBCN.TMA	48	1596	5	22	414	0	619	2	8	3
WIBCN.TFC	22	1653	18	83	578	5	346	2	3	6
WIBCN.TFA	15	1898	7	125	433	1	217	2	5	8
SPBCN.TMC	457	1525	8	80	327	39	219	12	2	41
SPBCN.TMA	638	1049	5	53	252	33	640	33	0	10
SPBCN.TMS	637	1224	8	89	258	38	411	19	1	27
SPBCN.TFC	175	1494	6	47	372	2	477	129	0	9
SPBCN.TFA	168	1471	7	17	224	2	504	312	1	2
SPBCN.TFS	197	1271	5	21	177	4	762	271	1	0
SUBCN.CF	17	1583	2	364	290	0	420	12	1	23
SUBCN.AF	15	1441	10	397	493	0	315	13	1	14
SUBCN.SF	47	1716	15	312	328	0	263	14	3	8
SUBCN.C	121	1650	3	412	275	3	214	18	2	5
SUBCN.A	547	1400	41	263	251	1	194	4	5	2
SUBCN.S	482	1425	20	241	298	0	223	2	2	7
SPBLA.TMC	952	1428	5	66	142	4	94	2	9	4
SPBLA.TMA	877	1447	4	51	117	4	186	4	18	5
SPBLA.TMS	911	1507	2	64	131	2	80	1	10	3
SPBLA.TFC	1225	1206	3	96	116	1	52	0	16	0
SPBLA.TFA	440	1898	4	163	113	1	76	0	14	1
SPBLA.TFS	625	1697	5	166	144	1	62	0	11	6
SUBLA.C	470	1652	1	245	255	5	71	2	0	13
SUBLA.A	548	1582	1	253	245	3	69	7	3	3
SUBLA.S	752	1449	1	214	249	1	32	6	2	4
SUOFF.C	476	1733	0	113	334	2	36	2	8	3
SUOFF.A	237	1697	0	84	638	2	41	1	8	2
SUOFF.S	649	1677	0	106	227	0	38	1	4	7

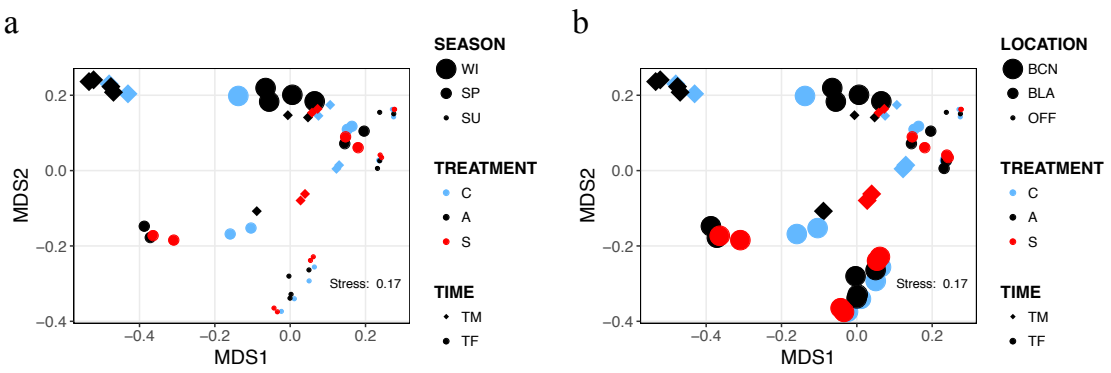

Figure S5.1. Non-metric multidimensional scaling (NMDS) representation of bacterial communities from the six experiments. a) Samples are categorized according to location, aerosol type, and sampling time. b) Same as in (a) but different sizes represent now different seasons. NMDS ordination was derived from pairwise Bray–Curtis distances. Each symbol corresponds to one sample ($N = 58$; see Table 5.1).

Chapter 6. General discussion and perspectives

Despite the importance of certain nutrients derived from the atmospheric particles such as nitrogen (N), phosphorous (P), silicate or iron in marine biogeochemical cycles, atmospheric deposition fluxes of these nutrients in the ocean remain poorly quantified, and are often inferred from airborne particle concentration measurements. Impact studies have mostly targeted effect of natural mineral aerosols on phytoplankton dynamics, and normally do not measure deposition. About one third of the population in the Mediterranean lives in coastal areas and has a footprint on the aerosol composition, with a potential differential impact of deposition on marine microbial population and biogeochemical cycles.

In this thesis I have addressed some of these knowledge gaps by linking direct time series measurements of deposition at coastal stations suffering different anthropogenic footprints with detailed impact studies of aerosols of different origin, including mostly anthropogenic particles, on bacterial and phytoplankton microbial dynamics. The following sections of this discussion will target some overarching aspects of the results of this thesis.

6.1 Atmospheric deposition does not match particle concentrations in air

Results from Chap. 2 highlight the need to measure the atmospheric fluxes of nutrients instead of estimating them from atmospheric particle concentrations and settling velocities. We found no correlation between deposition fluxes and the particle air concentration of any of the studied nutrients (nitrate, nitrite, ammonium, phosphate, silicate and organic carbon). It is known that particle settling velocity may vary with aerosol quality and meteorological conditions (Gelado-Caballero et al., 2012; López-

García et al., 2013). We did not find significant correlations between nutrient deposition fluxes and total PM_{10} or PM_{10} of Saharan origin either. Nitrate, nitrite, organic carbon and to a lesser extent phosphate and silicate followed seasonal cycles. Silicate and phosphate were correlated to rain in principal component analyses, probably indicating a washing out of atmospheric particles and an increased solubility in rainwater, as previously observed (Koçak, 2015; Ridame and Guieu, 2002; Ternon et al., 2010). Atmospheric deposition of Saharan origin in the northwestern Mediterranean is totally event driven and the largest modeled deposition fluxes occur under wet deposition (Gallisai et al., 2016). For most nutrients, high measured deposition fluxes sometimes coincided with large dust events. Nitrite deposition was higher in autumn and winter, while it was higher during spring and summer in the case of nitrate and silicate. P deposition was higher during summer-fall, coinciding also with the stratification period of the water column. In particular, the pattern of nitrate does not seem to be related to particulates but to reactions of ammonia gas with seawater (Viana et al., 2005). The deposition flux measurement station in Barcelona and the air particle concentration measurement station are 7 km apart. It is possible that some differences between deposition and air quality are affected by high spatial heterogeneity, but differences in seasonal patterns could hardly be explained by such heterogeneity. If spatial heterogeneity is very local, the number of measurement stations will have to be increased significantly for measurements to be accurate and be able to feed models.

6.2 Effect of atmospheric deposition on marine planktonic microorganisms

Our results from six experiments performed in three locations of the NW Mediterranean, impacted to a different degree by human activities − from a pristine environment offshore the Balearic Islands to a highly impacted urban location

(Barcelona), − and carried out at different times of the year (in Barcelona and Blanes), evidence the positive impact of atmospheric particles on chlorophyll (Chl) and bacterial activity during the stratification period (spring and summer). These results are in agreement with the correlation found between the atmospheric deposition fluxes of certain nutrients and Chl and bacterial production (BP) in Blanes (Chap. 2). To drive general patterns is still difficult, though. Fig. 6.1 shows our results and data from the literature in terms of Chl, bacterial abundance (BA) and BP with respect to the concentration of aerosols added (as a proxy of the release in N and P), and the concentration of aerosols added normalized by the existing Chl in water (as a proxy for the degree of oligotrophy in the receiving waters). We see an increase in Chl and BP with increasing concentration of aerosol, while no clear pattern is found for BA (Fig. 6.1, left panels). Fig. 6.1 (right panels) also evidences the importance of the trophic status of seawater (related to the time of the year as well) when assessing the effect of the aerosols. In this case, the stimulation of Chl and BP show opposing trends. When the system is poor in chlorophyll, BP increases, while the response of Chl to aerosols decreases. These results agree with those of Marañón et al. (2010). They also agree with recent interpretations of chlorophyll response to large dust events over the MS (Gallisai et al., 2016), where the chlorophyll in the eastern Mediterranean with ultraoligotrophic conditions and larger aerosol inputs shows less response than in the western Mediterranean. It seems that because of the ultraoligotrophic conditions, heterotrophic bacteria outcompete phytoplankton after deposition inputs, due to their larger surface:volume ratio (Raven, 1998).

Overall, we found that the effect on bacterial abundance was small, as observed in previous experiments where Saharan dust was used to amend microcosms (Herut et al.,

2005; Laghdass et al., 2011; Lekunberri et al., 2010; Marañón et al., 2010) or mesocosms in the western Mediterranean (Pulido-Villena et al., 2014). Studies in which the effect of anthropogenic particles on marine prokaryotes has been assessed are scarcer, but results also tend to show a negligible effect on bacterial abundance (Bonnet et al., 2005; Martínez-García et al., 2015; Teira et al., 2013; Ternon et al., 2011) compared to bacterial production (Herut et al., 2016; Martínez-García et al., 2015; Teira et al., 2013). To our knowledge, the study carried out by Herut et al. (2016) in the eastern MS is the only in which the effect of both dust and anthropogenic aerosols on phytoplankton and bacterial activity has been assessed so far. In agreement with these authors, we also found a larger biological response with anthropogenic particles compared to dust, which in our case was mainly observed for Chl, BP, and enzymatic activity. Herut et al. (2016) observed that the effect on BP was higher than on primary production, aerosols favoring the heterotrophic metabolic processes, as also observed by Guieu et al. (2014) in the western Mediterranean. In contrast, in Barcelona, aerosols benefited more the growth of chlorophyll than that of heterotrophs in summer (when the studies of Herut et al. 2016 and Guieu et al. 2014 were performed), as observed in other less oligotrophic regions (Duarte et al., 2006, 2004; Martínez-García et al., 2015). In winter instead, we found a tendency to favor the growth of bacteria over phytoplankton. The effect of aerosols seems to be greater on metabolic processes, though (compare the scales in Fig. 6.1), and we neither measure primary production nor bacterial respiration, so we are not able to predict the final balance with respect to the carbon flux. However, we found a significantly higher enhancement in the activities of aminopeptidase (AMA) and ß-Glucosidase with anthropogenic particles. Both ectoenzymes are mainly produced by heterotrophic bacteria (Sala et al., 2001, and references therein), and constitute their initial response to environmental changes (Karner and Rassoulzadegan, 1995). Thus, the

study of extracellular enzyme activity provide useful information about the metabolic state of the community with respect the nutrient concentration in the seawater. In spite of that, only Krom et al. (2016) evaluated the effect of alkaline phosphatase (APA) after aerosol additions in the eastern Mediterranean, and found a decrease in this enzyme due to the P released with the particles. APA is mostly synthetized by algae, and we did not find a significant stimulation for this enzyme in any of the aerosol treatments with respect to the controls (see Chap. 4 for further information). Thus, results from enzymatic activities also point towards the effect of (anthropogenic) aerosols being higher on the heterotrophic metabolism. It must also be mentioned that most of the previous aerosol-amendment studies were performed during the oligotrophic season, what does not allow establishing an effect of aerosols at annual scales. We here made a preliminary effort to assess the effect of aerosols in coastal locations at an annual scale, but we still only have results from one experiment carried out during the mixing period (winter) in one of the locations. Hence, more studies that evaluate the combined effect of different types of atmospheric particles on the whole planktonic community are needed to further assess the role of aerosols on the net flux of carbon. A further consideration is the effect of aerosols as catalyzers for aggregate formation, a process that has been observed to be an important pathway of particulate organic carbon export to deep waters in regions subjected to high dust deposition fluxes and low productivity such as the MS (Louis et al., 2017; Ternon et al., 2010; Bressac et al., 2014). The efficiency of this process could counteract the predominance of the respiration process over primary production by sequestering CO_2 in the deep ocean.

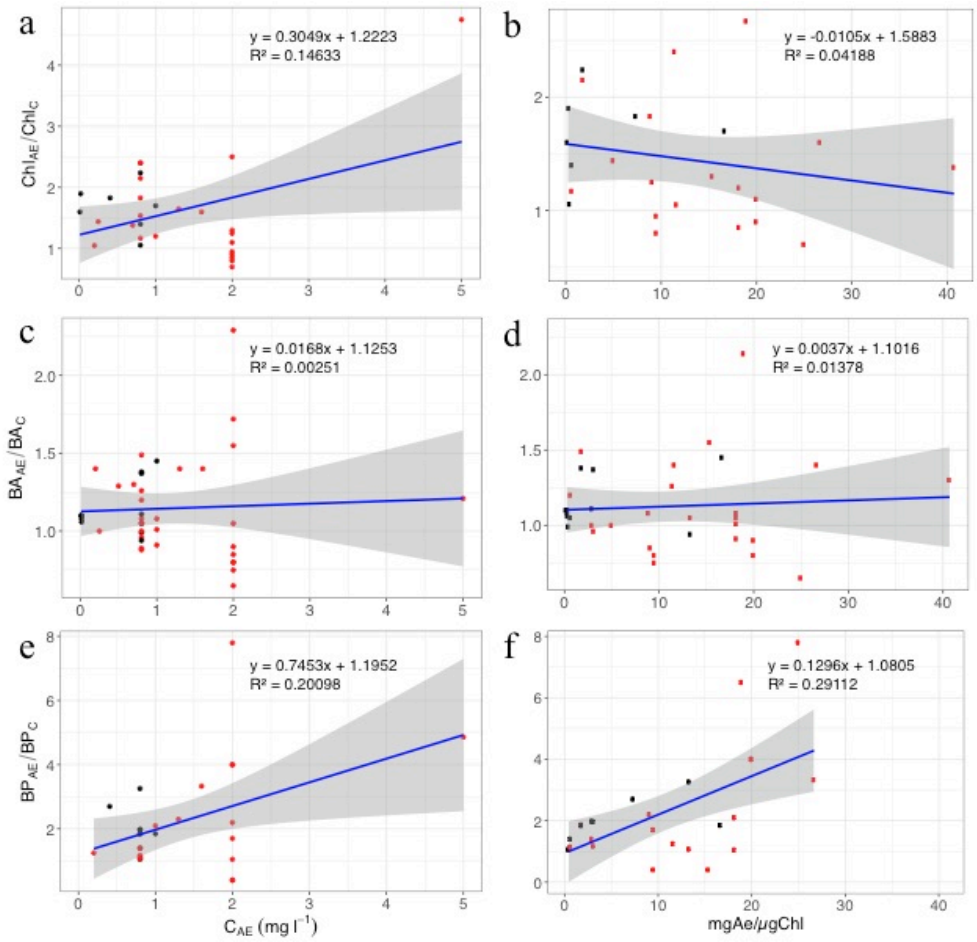

Figure 6.1. Response of Chl (a, b), BA (c, d) and BP (e, f) to aerosol amendments with respect to controls − without amendment − plotted against the aerosol concentration added into the seawater (left panels) and the aerosol concentration divided by the initial concentration of chlorophyll in the water (right panels). The grey shading delimits the confidence interval (95%). The data is from studies performed in the MS (Table 1.1 in Chap. 1), from this thesis, and from two studies carried out in the Atlantic Ocean (Duarte et al., 2006; Marañón et al., 2010). Black and red dots represent the effect of anthropogenic and Saharan aerosols, respectively. Note that most of the data come from studies where a particle concentration of 0.8 mg l^{-1} (Laghdass et al., 2011; Pulido-Villena et al., 2014; This study) or 2 mg l^{-1} (Marañón et al. 2010) was added, and there is only one data for aerosol concentration > than 2 mg l^{-1} (Herut et al., 2005), which makes the correlations very uncertain for values above this threshold.

On the other hand, aerosols favored the growth of certain groups of bacteria over other depending on the location and time of the year. As observed with the other biological variables, no significant effect was observed in winter, but major changes occurred in spring and summer in Barcelona and Blanes. In spring, anthropogenic aerosols favored more the growth of *Alphaproteobacteria* (mainly from the order *Rhodobacterales*, that

seemed to be the main contributors to bacterial production), *Flavobacteria* and *Sphingobacteria* (both belonging to the phylum *Bacteroidetes*), whereas Saharan dust enhanced more the growth of *Cyanobacteria* during summer in Blanes. In Barcelona, in summer, both types of particles stimulated the growth of *Alphaproteobacteria* (again, mainly *Rhodobacterales*), *Betaproteobacteria*, *Cyanobacteria* and *Gammaproteobacteria* (*Alteronomadales*). Laghdass et al. (2011) and Marañón et al. (2010) also found an increase in *Gammaproteobacteria* following Saharan dust additions in the western Mediterranean and in the Atlantic Ocean, respectively. Teira et al. (2013) observed a positive response in *Betaproteobacteria*, *Bacteroidetes* and *Gammaproteobacteria* after rainwater additions collected in an urban location in the NW Iberian Peninsula, and Guo et al. (2016) found that *Alphaproteobacteria* were the main group of the active fraction of bacteria directly stimulated by aerosols (especially when of anthropogenic origin) in the Eastern Mediterranean, thus increasing bacterial production. These authors also observed a later increase in *Gammaproteobacteria*, attributed to the increase in phytoplankton biomass and not to a direct effect of aerosols. On the other side, we observed a decrease in SAR11 (a group belonging to *Alphaproteobacteria*) with anthropogenic aerosols and SAR86 (belonging to *Gammaproteobacteria*) with both types of atmospheric particles. These two groups are characteristic of the oligotrophic ocean and in particular SAR11 is considered to be the most abundant taxon in the whole ocean (Giovannoni and Vergin, 2012; Morris et al., 2002). A decrease in the abundance of SAR11 upon dust and anthropogenic aerosols additions has been previously observed (Guo et al., 2016; Hill et al., 2010; Marañón et al., 2010), and Nogales et al. (2007) observed that active SAR11 cells decreased in the most nutrient-enriched areas in the island of Mallorca. Hence, we can conclude that SAR11 and SAR86 are groups typical of oligotrophic waters and are less favored by

addition of nutrients from aerosols, which would benefit more other groups of *Alphaproteobacteria*, *Gammaproteobacteria* or *Bacteroidetes*, the final outcome depending on the initial status of the seawater and the nutrients released by aerosols.

Finally, it must be mentioned that aerosols are also a source of microorganisms to the surface ocean. It has recently been observed that some airborne bacteria may be released with the aerosols, with the potential to develop in the water and yielding changes in the community composition and the bulk rates of prokaryotic production and N_2 fixation (Peter et al., 2014; Rahav et al., 2016; Reche et al., 2009). However, this was unlikely to occur in our experiments, as aerosol samples were subjected to a cycle of freezing − sonication (20 min at 7 kHz) − freezing, processes that probably removed most of the airborne bacteria and spores or at least reduced highly their viability (Evelyn and Silva, 2015; Foladori et al., 2007).

6.3 What makes anthropogenic aerosols more appealing to prokaryotes?

Both Saharan dust and anthropogenic aerosols are known to have an N to P ratio much larger than the value of 16 obtained by Redfield (1963) for marine microorganisms. This ratio seems to be especially high for anthropogenic particles (Herut et al., 2016; Markaki et al., 2010, 2003; see also Annexes II and III). Results from our six experiments show that both types of aerosols were a source of N and, to a lesser extent, P. The N supplied by anthropogenic aerosols was significantly higher than that released by Saharan dust (see Table 4.1 in Chap. 4). In addition, the supply of organic carbon was also higher with anthropogenic particles, but it usually remained at high concentrations during the incubation period. We also found a negligible utilization of chromophoric compounds during the experiments (Sánchez-Pérez et al., 2016),

suggesting that the organic carbon supplied by aerosols may be mostly recalcitrant, as previously reported (e.g., Duarte et al., 2006). Small labile fractions of the organic matter that stimulate bacterial activity but can not be detected in bulk TOC trends are not discarded. The higher release of inorganic N and P, as well as organic carbon with aerosols, is the result of not only higher loads of these compounds within anthropogenic particles, but also because they are richer in NOx and SOx compounds, the main acid precursor species in the atmosphere (Stockdale et al. 2016), thus favoring the dissolution of the compounds within these particles, making them more bioavailable once released into the seawater (see Chapters 3 and 4 for a further discussion about the amount of nutrients released from both types of aerosols).

In all, in contrast to previous studies that have suggested that P was the main constituent of dust that triggered a response in the microbial planktonic communities of the western MS (Lekunberri et al., 2010; Pulido-Villena et al., 2014, 2008; Reche et al., 2009; Romero et al., 2011), our results show that both N and P are the main drivers in the stimulation of the heterotrophic metabolism in coastal waters of the western Mediterranean, the importance of one or another depending on the initial biogeochemical status of the seawater. Furthermore, the additional N released by anthropogenic aerosols could be the responsible of the major enhancement observed overall with these particles. In agreement, Herut et al. (2016) found that anthropogenic aerosols in the eastern MS were enriched in nitrate compared to bioavailable phosphate, and that it was this extra N the main driver of the larger response observed in most of the biological variables with anthropogenic aerosols compared to Saharan dust. However, it must be considered that, albeit P is the main limiting nutrient in the WMS (e.g., Lazzari et al., 2016; Marty and Chiaverini, 2002), we observed an initial co-

limitation of N and P before the aerosol additions at most of the experiments (N:P ratio < 16). The exceptions were the winter and summer experiments carried out in Barcelona, where the N:P ratio was much larger than 16. In the first case, almost no response in any of the biological variables was detected, whereas in summer a higher release of P from aerosols compared with the other experiments was determined, and a significant positive correlation between this P and the response of some biological variables was found. Therefore, overall, we can conclude that the effect of aerosols on marine planktonic microorganisms in coastal waters is dependent on three factors: (1) the initial status of the seawater; (2) the chemical composition of the atmospheric particles; and (3) the solubility of the atmospheric particles into the seawater.

6.4 Aerosols directly affect prokaryotes

There is a discussion about whether atmospheric particles can directly impact marine bacteria or there is a mediated effect through phytoplankton growth. Results reported so far have determined a direct effect in some experiments where the effect on bacteria was assessed without the presence of competitors and predators (Pulido-Villena et al., 2008), and a quicker response of bacteria during very oligotrophic conditions (Guieu et al., 2014b; Herut et al., 2016; Marañón et al., 2010). Our results from the stronger positive correlation found between N and P deposition and BA and BP in the coasts of Barcelona and Blanes, respectively, compared to that with Chl (Chap. 2), as well as from the significantly higher stimulation of all the prokaryotic parameters with aerosols in the filtered microcosms (Chap. 4), confirm previous results.

6.5 Relevance to the natural environment

The concentration of atmospheric particles added (0.8 mg l^{-1}) is equivalent to a medium-high Saharan dust deposition event of 8 g m^{-2} into a mixed layer depth of 10 m, which is approximately the depth of the thermocline during the stratification period in the NW Mediterranean (Ridame and Guieu, 2002; D'Ortenzio et al., 2005). This concentration is within the range used in previous Saharan dust-enrichment bioassays carried out in the Mediterranean (e.g., Guieu et al., 2010; 2014a; Herut et al., 2016, 2005; Pulido-Villena et al., 2008). Thus, our additions are realistic for Saharan dust deposition events. Anthropogenic particles account for more than 90% of the particulate matter in the atmosphere of Barcelona and Blanes (see Chap. 2), however, anthropogenic deposition events are about one order of magnitude lower in the NW Mediterranean (Izquierdo et al., 2012; Pulido-Villena et al., 2008). In this study, though, we aimed to compare the effect of anthropogenic aerosols with Saharan dust under equal concentrations (in terms of dry weight), and similar concentrations of anthropogenic particles have been used in previous addition experiments (Herut et al., 2016; Paytan et al., 2009). Furthermore, anthropogenic atmospheric fluxes are still expected to increase globally (Christodoulaki et al., 2016; Duce et al., 2008; IPCC, 2014), and especially in coastal areas due to the high demographic pressure (Lionello et al., 2006).

Albeit the results presented in this thesis contribute to assess the effect of aerosols into the marine environment, it is difficult to extrapolate the real impact at basin scale or to other oligotrophic regions. Results from Chap. 2 show that atmospheric-derived N, P and Si in the western basin would contribute to 38%, 15% and 0.72% of the total new primary production, respectively. Atmospheric N and P could account, on the other

hand, for up to 68% and 16% of bacterial production. Results from both the correlation between the atmospheric data and the biological variables measured in the coastal waters of Barcelona and Blanes, and from the microcosm experiments, tend to show that atmospheric N and P released from the atmosphere would mainly benefit the heterotrophic planktonic community instead the primary producers. It has been suggested that heterotrophy is favored over autotrophy during incubation experiments due to the "bottle enclosure effect" (Calvo-Díaz et al., 2011; Duarte and Agustí, 1998; Williams et al., 2004). Calvo Díaz et al. (2011) found that the ratio of autotrophic:heterotrophic prokaryotes biomass decreased inside experimental bottles of volume < 1 L during incubation times of 24 h. However, the containers that we used were much larger (15 L), and the incubation time much longer (≥ 48 h), diminishing the "bottle effect". Nevertheless, in our microcosm experiments, we are comparing the results observed in the amended containers (A, S) with respect the controls, which would be affected in the same way by the enclosure.

The increase in aridity already observed in the Mediterranean region (IPCC, 2014; Prospero and Lamb, 2003) combined with a more acidic atmosphere over the MS (Stockdale et al., 2016) are expected to increase the amounts of available N, P, Si, organic compounds and trace metals released by the atmosphere into surface waters of the Mediterranean. On the other hand, a decrease in the two other main sources of nutrients to the basin, winter convection and river discharges, is expected as a consequence of the increase in the thermal stratification (Lionello et al., 2006) and the decrease in precipitation (Durrieu de Madron et al., 2011; IPCC, 2014), respectively. The effect of nutrients released from the atmosphere in the planktonic communities of the MS and other oligotrophic aquatic environments affected by high atmospheric

inputs from both mineral and anthropogenic sources (e.g., the Red Sea, the North Atlantic, the Caribbean, or some inland lakes) is therefore becoming increasingly important. Because physical (i.e. the relatively high temperature of the water) and biological (i.e. the predominance of heterotrophy over autotrophy in oligotrophic environments) processes, these areas already tend to be a source of CO_2 instead a sink. Hence, if heterotrophy is generally favored by atmospheric deposition, these areas would tend to increase even more the release of CO_2, at least during the warm season, when the seawater temperature is higher and stratification favors the impact of atmospheric particles.

Conclusions

The main conclusions reached in this thesis are the following:

1) The deposition of nutrients from the atmosphere (nitrate, nitrite, ammonium, phosphate, silicate, and organic carbon) in coastal locations of the northwestern Mediterranean Sea is not correlated with the concentration of either total particle in the atmosphere or dust particles. Rainfall seems to drive phosphorus and silicate deposition. Nutrient deposition fluxes are higher in coastal locations with higher anthropogenic footprint than in less human-impacted areas.

2) Comparing the effect of mineral and anthropogenic aerosols in the same coastal location at different times of the year, both types of particles yield a mayor stimulation of chlorophyll and bacterial abundance and production during the stratification period (i.e. late spring and summer), whereas their effect in winter is negligible. Anthropogenic particles favored more the growth of certain groups of phytoplankton (especially nanoeukaryotes), while bacterial abundance was more positively affected by Saharan dust. The overall effect is determined by the initial trophic status of the seawater and the concentration of nutrients released by the aerosols.

3) Comparing the effect of mineral and anthropogenic aerosols in locations with different anthropogenic footprint at different times of the year, prokaryotic activity was more stimulated with anthropogenic aerosols, especially during most oligotrophic conditions (i.e. in summer and in the more pristine environment). This is attributed to the additional N released by the anthropogenic particles at times of the year when both N and P co-limit the plankton production.

4) Both anthropogenic aerosols and Saharan dust produced a more positive effect on bacterial abundance, production, and enzymatic activity in the absence of competitors and predators, showing that impact on bacteria do not need an established food web.

5) Aerosols yielded changes in the bacterial community composition at given locations and times of the year. Both atmospheric particles produced a positive effect on *Gammaproteobacteria* of the order *Alteromonadales* and a negative effect on *Oceanospirillales* (SAR86). They also stimulated the growth of *Cyanobacteria* during summer, especially the Saharan dust. Anthropogenic aerosols instead favored more the growth of certain groups of *Alphaproteobacteria* (*Rhizobiales*, *Rhodobacterales* and *Rickettsiales*), *Flavobacteria* and *Sphingobacteriales*, especially during spring. No significant changes in the bacterial structure were observed during winter.

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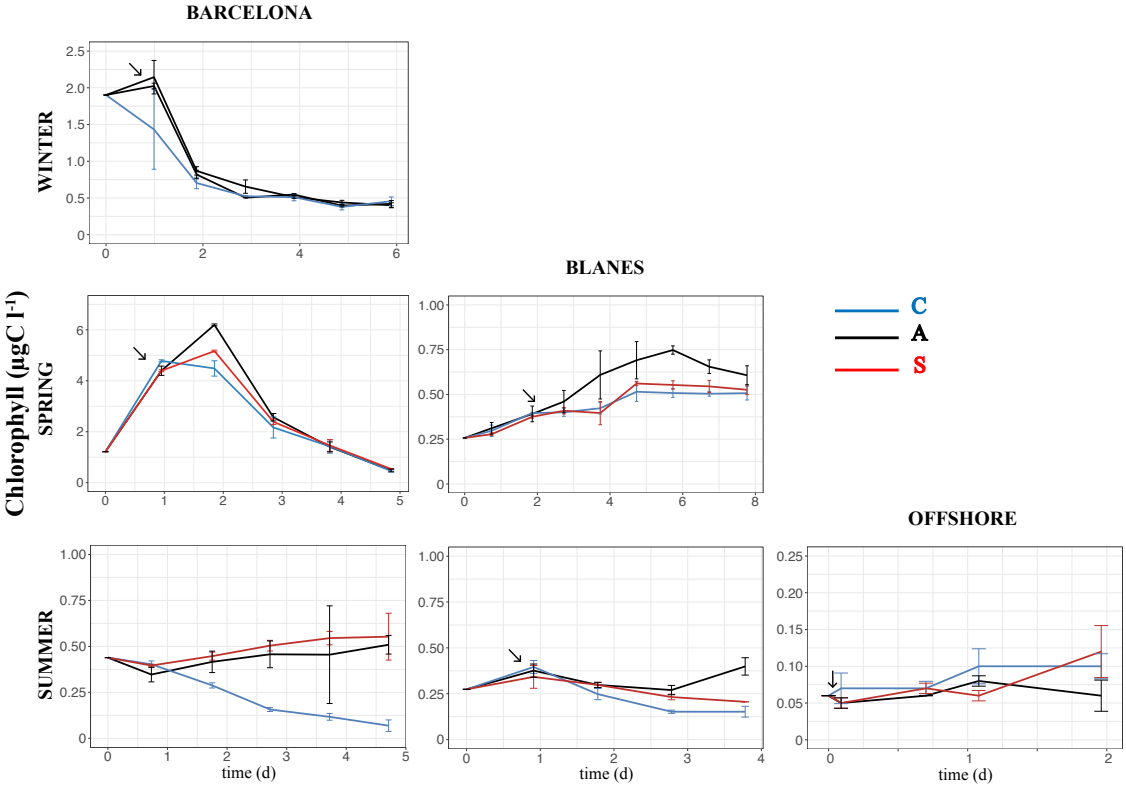
Annex I: Chlorophyll



Dynamics of chlorophyll (µg C l^{-1}) over the incubation time in the six experiments. Error bars represent the standard error from two replicate containers. Arrows point the moment of aerosol additions. C = controls; A = anthropogenic; S = Saharan. In winter, there were no Saharan containers, but four anthropogenic microcosms were amended with anthropogenic particles of differnet conmposition (see Sect. 1.7 in Chap. 1).

Annex II: Chemical composition of the collected filters

Table AII1. Chemical composition of the filters used in Barcelona in the experiments of winter (WI-BCN), spring (SP-BCN) and summer (SU-BCN). TSP = total suspended particles.

	WI-BCN			SP-BCN			SU-BCN					
µg/m3	F2 (A1)	F3 (A1)	F1 (A2)	F10 (A)	F11 (A)	F12 (S)	F3 (A)	F8 (A)	F13 (A)	F6 (S)	F10 (S)	F11 (S)
Date	15.01.14	17.01.14	17.12.14	27.01.13	17.02.14	18.02.14	10.04.13	04.07.13	12.08.13	07.06.13	05.08.13	05.08.13
TSP	41.70	41.70	83	20.8	41.7	180.7	69.5	124.0	152.9	190.6	90.3	69.5
OC	6.39	7.87	17.34	4.39	7.43	9.59	13.65	8.90	8.00	9.00	6.90	5.40
EC	2.41	3.25	7.84	1.97	2.71	2.61	2.91	2.20	2.30	0.90	1.90	1.50
CO3	2.62	3.84	5.02	2.76	3.57	8.15	9.80	9.50	9.90	17.90	5.70	3.90
SiO2	1.86	2.41	5.16	2.59	5.89	50.36	12.95	45.00	48.60	88.60	22.30	15.30
Al2O3	0.62	0.80	1.72	0.86	1.96	16.79	4.32	15.00	16.20	29.50	7.40	5.10
Ca	1.74	2.56	3.34	1.84	2.38	5.43	6.53	6.30	6.60	12.00	3.80	2.60
Fe	1.04	1.25	2.30	0.96	1.25	5.96	2.18	5.10	5.40	9.40	2.40	1.70
K	0.23	0.81	0.67	0.26	0.68	2.63	1.92	3.10	3.40	6.10	1.70	1.20
Na	0.42	2.49	2.48	0.70	1.10	6.93	2.93	6.80	3.80	14.30	3.20	2.10
Mg	0.20	0.45	0.56	0.26	0.41	1.90	1.16	2.50	2.20	5.20	1.30	0.80
SO42-	1.03	1.51	3.63	0.92	1.70	4.04	3.26	3.90	4.30	5.90	5.80	3.50
NO3-	1.70	3.38	9.91	0.77	4.15	3.82	4.07	4.80	5.90	6.30	3.70	3.30
Cl-	0.27	3.89	3.40	0.85	1.16	9.49	3.73	5.40	2.30	16.00	1.70	0.90
NH4+	0.30	0.52	2.81	0.13	0.80	0.94	0.20	0.30	0.50	0.20	0.40	0.30
P	0.03	0.05	0.08	0.03	0.06	0.13	0.10	0.14	0.14	0.28	0.09	0.06

ng/m3												
Li	0.40	0.55	0.85	0.47	0.84	5.38	2.20	5.26	5.32	11.01	2.50	1.78
Be	0.05	0.05	0.07	0.03	0.07	0.34	0.08	0.20	0.21	0.41	0.10	0.08
B	5.24	22.91	<dl	< dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl
Sc	0.06	0.09	0.16	0.10	0.20	1.21	0.50	1.48	1.59	2.88	0.67	0.46
Ti	35.84	52.37	125.91	43.90	85.34	781.31	167.04	404.77	425.26	770.90	208.93	143.08
V	2.99	9.20	18.92	4.14	14.86	19.03	19.02	27.98	26.50	49.40	24.61	18.52
Cr	8.91	19.48	16.25	5.12	11.38	15.78	36.76	41.53	34.80	98.97	28.35	25.86
Mn	23.94	24.72	36.84	27.13	24.80	109.14	54.19	146.29	128.04	273.28	57.22	38.40
Co	0.27	0.40	0.84	0.25	0.67	2.48	0.84	1.46	1.46	2.65	1.02	0.71
Ni	2.19	4.60	5.63	2.13	11.06	8.56	28.66	12.64	8.92	31.35	15.61	11.49
Cu	52.16	49.83	118.26	40.19	49.56	42.44	50.31	65.01	55.24	45.19	54.45	33.21
Zn	160.89	229.68	230.63	126.04	182.31	146.56	240.57	288.53	193.18	649.49	228.69	186.93
Ga	0.15	0.18	0.31	0.17	0.33	2.86	0.76	2.21	2.29	4.16	1.07	0.75
Ge	0.80	0.76	0.31	0.14	0.67	0.86	0.85	0.59	0.69	0.09	0.50	0.75
As	0.42	2.12	1.08	0.45	0.64	1.70	1.73	3.12	3.13	5.71	1.40	0.99
Se	0.09	0.45	0.57	0.10	0.56	0.44	0.61	1.00	0.60	1.01	0.59	0.41
Rb	0.74	1.20	1.74	0.89	1.92	11.80	5.06	14.73	15.33	27.24	6.43	4.44
Sr	4.33	6.86	8.35	4.76	7.50	38.06	16.53	34.42	32.98	67.36	17.33	12.12
Y	0.55	0.67	0.28	0.20	0.79	2.21	0.98	2.46	2.62	5.13	1.39	1.00
Zr	20.34	22.96	29.15	2.13	4.88	40.17	17.76	65.87	65.46	167.06	34.20	30.47
Nb	0.90	0.98	1.83	1.00	1.58	18.35	2.57	5.92	6.13	11.60	2.92	2.35
Mo	23.92	28.32	8.37	0.86	18.54	18.73	12.61	14.21	10.89	57.66	14.33	9.75
Cd	0.14	0.36	0.42	0.21	0.28	0.25	0.23	0.41	0.16	0.40	0.14	0.12
Sn	7.56	7.98	16.52	4.28	9.31	5.94	12.76	8.16	9.19	7.86	4.53	3.68
Sb	4.92	5.14	12.16	3.28	4.87	8.02	5.72	5.05	5.06	4.23	3.12	2.68
Cs	0.07	0.11	0.14	0.07	0.17	0.63	0.25	0.71	0.71	1.20	0.33	0.23
Ba	85.41	100.70	127.07	16.63	96.89	147.89	36.30	274.70	214.01	644.78	223.53	146.41

La	0.41	0.61	0.76	0.44	0.86	5.23	1.65	4.18	4.59	7.77	2.35	1.68
Ce	0.88	1.09	1.64	0.89	1.63	11.72	2.86	7.63	8.36	14.14	4.16	2.96
Pr	0.07	0.09	0.13	0.08	0.16	1.37	0.34	0.93	1.02	1.80	0.47	0.34
Nd	0.26	0.32	0.42	0.30	0.58	4.81	1.20	3.33	3.66	6.45	1.71	1.23
Sm	0.07	0.09	0.08	0.06	0.14	1.00	0.28	0.76	0.83	1.51	0.40	0.28
Eu	0.02	0.02	0.03	0.01	0.03	0.19	0.05	0.14	0.14	0.27	0.08	0.05
Gd	0.09	0.12	0.08	0.06	0.18	0.90	0.26	0.74	0.80	1.48	0.42	0.31
Tb	0.02	0.03	0.02	0.01	0.03	0.14	0.04	0.11	0.12	0.24	0.07	0.05
Dy	0.12	0.17	0.06	0.05	0.17	0.67	0.23	0.64	0.69	1.37	0.39	0.29
Ho	0.02	0.03	0.01	0.01	0.03	0.14	0.04	0.12	0.13	0.25	0.07	0.05
Er	0.06	0.07	0.04	0.03	0.08	0.39	0.11	0.33	0.37	0.70	0.19	0.14
Tm	0.01	0.01	<dl	<dl	0.01	0.05	0.02	0.05	0.05	0.10	0.03	0.02
Yb	0.04	0.05	0.03	0.03	0.07	0.36	0.10	0.31	0.34	0.63	0.18	0.14
Lu	0.01	0.01	<dl	<dl	0.01	0.05	0.01	0.05	0.05	0.10	0.03	0.02
Hf	0.93	1.06	1.33	0.08	0.17	2.08	0.71	2.15	2.16	5.25	0.94	0.85
Ta	0.09	0.07	0.11	0.06	0.12	3.93	0.06	0.43	0.50	0.55	0.08	0.11
W	1.13	1.45	6.38	1.65	3.10	3.59	0.50	2.62	2.35	4.60	1.53	1.00
Tl	<dl	0.07	0.09	0.03	0.06	0.10	0.09	0.23	0.14	0.23	0.08	0.06
Pb	7.47	13.03	23.17	11.09	12.92	14.50	17.81	34.82	17.39	30.22	10.10	7.19
Bi	0.48	0.55	1.65	0.28	1.20	0.41	0.51	0.72	0.69	0.68	0.33	0.29
Th	0.21	0.27	0.24	0.12	0.30	1.06	0.61	1.80	2.00	3.73	0.96	0.75
U	0.30	0.38	0.14	0.06	0.22	0.63	0.21	0.61	0.63	1.51	0.53	0.41

Table AII2. Chemical composition of the filters used in Blanes in the experiments of spring (SP-BLA) and summer (SU-BLA), and in the experiment "offshore" (SU-OFF). TSP = total suspended particles.

	SP-BLA					SU-BLA			SU-OFF		
µg/m3	F4 (A)	F5 (A)	F6 (A)	F8 (A)	F9 (S)	F14 (A)	F16 (A)	F9 (S)	F2 (A)	F17 (A)	F14 (S)
Date	21-23.01.14	11-13.02.14	25-27.01.14	18-20.03.14	18-20.02.14	06-09.05.14	02-05.06.14	18-20.02.14	22-23/07/14	28-29/07/14	01-03/04/14
TSP	13.9	17.4	10.43	44.03	117.5	44.03	32.44	117.5	111.23	69.52	177.19
OC	4.91	3.65	5.79	8.54	5.00	7.87	4.35	5.00	4.66	5.58	13.56
EC	0.77	0.66	0.60	1.60	0.78	0.92	0.76	0.78	0.91	1.07	2.52
CO3	0.32	0.80	0.44	1.54	4.74	1.26	1.14	4.74	5.46	2.81	16.19
SiO2	0.36	1.13	0.52	2.89	47.74	2.58	2.07	47.74	24.72	6.71	32.59
Al2O3	0.12	0.38	0.17	0.96	15.91	0.86	0.69	15.91	8.24	2.24	10.86
Ca	0.21	0.53	0.29	1.03	3.16	0.84	0.76	3.16	3.64	1.87	10.80
Fe	0.10	0.19	0.14	0.39	4.41	0.37	0.29	4.41	2.56	0.87	4.13
K	0.19	0.22	0.23	0.50	1.90	0.36	0.23	1.90	1.70	0.74	2.29
Na	0.48	1.02	0.38	1.41	1.76	1.60	1.21	1.76	2.65	6.39	6.24
Mg	0.06	0.17	0.09	0.25	1.43	0.30	0.23	1.43	1.12	1.01	2.49
SO42-	1.13	0.78	0.43	4.11	1.92	2.40	2.56	1.92	1.40	1.86	9.53
NO3-	2.18	1.27	0.86	6.15	2.91	3.08	1.44	2.91	2.15	1.19	10.33
Cl-	0.24	1.00	0.14	0.42	1.38	1.02	0.77	1.38	2.92	10.17	10.01
NH4+	0.50	0.16	0.09	0.77	0.44	0.44	0.43	0.44	0.24	0.16	1.03
P	0.01	0.02	0.04	0.06	0.09	0.05	0.03	0.09	0.06	0.04	0.14
ng/m3											
Li	0.08	0.15	0.10	0.40	4.20	0.29	0.25	4.20	2.33	0.80	3.88

Be	0.02	0.03	0.02	0.04	0.24	0.02	0.02	0.24	0.12	0.05	0.20
B	<dl	<dl	< dl	< dl	< dl	< dl	< dl	<dl	< dl	< dl	< dl
Sc	0.01	0.04	0.02	0.10	1.43	0.00	0.00	1.43	0.62	0.00	1.12
Ti	6.39	17.33	9.58	33.34	549.35	29.77	24.00	549.35	222.97	66.91	400.20
V	1.24	1.40	0.87	10.72	13.73	9.12	5.47	13.73	11.64	4.39	28.17
Cr	1.36	1.41	0.32	1.50	8.60	9.12	0.96	8.60	12.01	8.29	22.27
Mn	5.68	5.92	5.46	12.05	74.84	8.49	6.77	74.84	94.37	26.64	70.89
Co	0.06	0.14	0.05	0.34	1.91	0.17	0.11	1.91	0.64	0.29	1.61
Ni	0.97	1.15	5.92	4.37	5.09	3.16	1.86	5.09	3.16	1.56	15.47
Cu	7.80	5.54	2.98	6.86	7.71	5.35	5.31	7.71	15.17	9.05	153.15
Zn	51.14	43.02	37.47	75.03	53.91	34.28	38.55	53.91	78.28	53.53	269.82
Ga	0.03	0.07	0.04	0.18	2.23	0.13	0.10	2.23	0.97	0.27	1.59
Ge	<dl	<dl	< dl	< dl	0.46	< dl	0.09	0.46	0.40	< dl	1.54
As	0.46	0.16	0.18	0.58	0.92	0.31	0.26	0.92	1.40	0.60	1.61
Se	0.13	0.09	0.09	0.84	0.33	0.51	0.51	0.33	0.22	0.36	1.48
Rb	0.33	0.44	0.34	1.13	8.92	0.80	0.62	8.92	6.48	1.86	7.74
Sr	1.01	1.89	1.10	3.74	24.03	2.49	2.17	24.03	16.59	8.82	35.48
Y	0.15	0.23	0.21	0.56	2.94	0.14	0.10	2.94	1.00	0.13	2.67
Zr	<dl	4.08	< dl	10.60	24.72	4.66	6.61	24.72	16.54	25.53	58.72
Nb	0.32	0.49	0.17	0.70	13.73	0.43	0.34	13.73	2.48	0.88	5.37
Mo	6.21	4.21	7.26	16.40	8.78	2.23	3.89	8.78	2.68	12.81	39.16
Cd	0.11	0.07	0.05	0.19	0.12	0.06	0.08	0.12	0.13	0.06	0.43
Sn	0.90	1.09	0.65	2.60	1.60	1.58	1.44	1.60	3.14	1.76	7.03
Sb	1.46	0.72	0.29	1.08	0.97	0.65	0.72	0.97	1.94	0.86	4.36
Cs	0.03	0.04	0.03	0.08	0.46	0.05	0.05	0.46	0.26	0.11	0.49
Ba	28.68	13.67	16.25	35.75	70.66	14.15	20.20	70.66	< dl	35.37	119.68
La	0.09	0.16	0.12	0.56	5.09	0.27	0.24	5.09	1.86	0.68	3.44
Ce	0.17	0.32	0.25	0.86	10.17	0.53	0.45	10.17	3.66	1.29	7.27

Pr	0.02	0.04	0.03	0.10	1.23	0.06	0.05	1.23	0.43	0.12	0.84
Nd	0.07	0.14	0.11	0.35	4.32	0.20	0.16	4.32	1.50	0.41	3.04
Sm	0.02	0.04	0.04	0.10	0.88	0.04	0.03	0.88	0.32	0.08	0.68
Eu	<dl	<dl	0.01	0.01	0.15	0.01	0.01	0.15	0.04	0.01	0.11
Gd	0.03	0.05	0.05	0.11	0.84	0.04	0.04	0.84	0.28	0.06	0.64
Tb	0.01	0.01	0.01	0.02	0.12	0.01	< dl	0.12	0.04	0.01	0.10
Dy	0.04	0.05	0.06	0.14	0.65	0.03	0.02	0.65	0.21	0.03	0.65
Ho	0.01	0.01	0.01	0.02	0.12	0.01	< dl	0.12	0.04	0.01	0.11
Er	0.02	0.03	0.02	0.06	0.35	0.02	0.01	0.35	0.12	0.02	0.30
Tm	<dl	<dl	0.00	0.01	0.05	< dl	< dl	0.05	0.02	< dl	0.04
Yb	0.02	0.02	0.02	0.05	0.32	0.02	0.01	0.32	0.11	0.03	0.29
Lu	<dl	<dl	0.00	0.01	0.05	0.01	0.01	0.05	0.03	< dl	0.04
Hf	<dl	0.21	< dl	0.45	1.29	0.16	0.23	1.29	0.62	1.00	2.61
Ta	0.01	0.05	< dl	0.11	2.34	0.01	< dl	2.34	0.11	< dl	0.67
W	0.26	3.83	0.10	0.35	4.57	0.19	0.04	4.57	1.20	0.38	1.51
Tl	<dl	<dl	0.00	0.07	0.07	0.02	0.02	0.07	0.05	< dl	0.09
Pb	5.30	2.78	1.95	7.21	5.76	2.79	3.34	5.76	11.06	3.54	15.66
Bi	0.33	1.33	0.10	0.18	0.11	0.10	0.13	0.11	0.25	0.08	1.21
Th	0.04	0.14	0.04	0.23	1.27	0.10	0.09	1.27	0.76	0.19	1.37
U	0.09	0.09	0.12	0.23	0.33	0.01	0.01	0.33	0.06	< dl	0.67

Annex III: Ratios between elements in the collected filters

Table AIII1. Ratios between chemical elements in the collected filters used in Barcelona in the experiments of winter (WI-BCN), spring (SP-BCN) and summer (SU-BCN).

	WI-BCN			SP-BCN			SU-BCN					
	F2 (A1)	F3 (A1)	F1 (A2)	F10 (A)	F11 (A)	F12 (S)	F3 (A)	F8 (A)	F13 (A)	F6 (S)	F10 (S)	F11 (S)
P/Al	0.056	0.062	0.046	0.032	0.029	0.008	0.024	0.009	0.008	0.010	0.013	0.012
Fe/Al	1.674	1.557	1.340	1.110	0.638	0.355	0.506	0.340	0.333	0.319	0.324	0.333
Pb/Al	1.20×10^{-2}	1.62×10^{-2}	1.35×10^{-2}	1.28×10^{-2}	6.58×10^{-3}	8.64×10^{-4}	4.13×10^{-3}	2.32×10^{-3}	1.07×10^{-3}	1.02×10^{-3}	1.36×10^{-3}	1.41×10^{-3}
Cd/Al	2.18×10^{-4}	4.51×10^{-4}	2.45×10^{-4}	2.45×10^{-4}	1.41×10^{-4}	1.51×10^{-5}	5.33×10^{-5}	2.73×10^{-5}	9.88×10^{-6}	1.36×10^{-5}	1.89×10^{-5}	2.35×10^{-5}
Zn/Al	2.59×10^{-1}	2.86×10^{-1}	1.34×10^{-1}	1.46×10^{-1}	9.28×10^{-2}	8.73×10^{-3}	5.57×10^{-2}	1.92×10^{-2}	1.19×10^{-2}	2.20×10^{-2}	3.09×10^{-2}	3.67×10^{-2}
Al/Ca	0.356	0.314	0.514	0.469	0.825	3.091	0.660	2.381	2.455	2.458	1.947	1.962
Al/Fe	0.597	0.642	0.746	0.901	1.568	2.816	1.977	2.941	3.000	3.138	3.083	3.000
Ca/Fe	1.678	2.047	1.451	1.921	1.900	0.911	2.994	1.235	1.222	1.277	1.583	1.529
Ti/Al	0.058	0.065	0.073	0.051	0.043	0.047	0.039	0.027	0.026	0.026	0.028	0.028
Ti/Ca	0.021	0.020	0.038	0.024	0.036	0.144	0.026	0.064	0.064	0.064	0.055	0.055
Ti/Fe	0.034	0.042	0.055	0.046	0.068	0.131	0.077	0.079	0.079	0.082	0.087	0.084

Table AIII2. Ratios between chemical elements in the collected filters used in Blanes in the experiments of spring (SP-BLA) and summer (SU-BLA), and in the experiment "offshore" (SU-OFF).

	SP-BLA					SU-BLA			SU-OFF		
	F4 (A)	F5 (A)	F6 (A)	F8 (A)	F9 (S)	F14 (A)	F16 (A)	F9 (S)	F2 (A)	F17 (A)	F14 (S)
P/Al	0.103	0.048	0.218	0.060	0.006	0.061	0.048	0.006	0.007	0.018	0.013
Fe/Al	0.873	0.509	0.787	0.401	0.277	0.429	0.423	0.277	0.311	0.388	0.380
Pb/Al	4.44×10^{-2}	7.38×10^{-3}	1.13×10^{-2}	7.49×10^{-3}	3.62×10^{-4}	3.25×10^{-3}	4.84×10^{-3}	3.62×10^{-4}	1.34×10^{-3}	1.58×10^{-3}	1.44×10^{-3}
Cd/Al	9.43×10^{-4}	1.96×10^{-4}	3.18×10^{-4}	1.96×10^{-4}	7.43×10^{-4}	7.44×10^{-5}	1.23×10^{-4}	7.43×10^{-6}	1.57×10^{-5}	2.73×10^{-5}	3.93×10^{-5}
Zn/Al	4.29×10^{-1}	1.14×10^{-1}	2.17×10^{-1}	7.80×10^{-2}	3.39×10^{-3}	3.99×10^{-2}	5.59×10^{-2}	3.39×10^{-3}	9.50×10^{-3}	2.40×10^{-2}	2.48×10^{-2}
Al/Ca	0.559	0.703	0.593	0.936	5.034	1.023	0.909	5.034	2.264	1.192	1.006
Al/Fe	1.145	1.964	1.271	2.494	3.606	2.329	2.365	3.606	3.214	2.578	2.631
Ca/Fe	2.049	2.792	2.145	2.663	0.716	2.276	2.602	0.716	1.420	2.162	2.615
Ti/Al	0.054	0.046	0.056	0.035	0.035	0.035	0.035	0.035	0.027	0.030	0.037
Ti/Ca	0.030	0.032	0.033	0.032	0.174	0.035	0.032	0.174	0.061	0.036	0.037
Ti/Fe	0.061	0.090	0.071	0.086	0.124	0.081	0.082	0.124	0.087	0.077	0.097