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Nematodes stimulate biomass accumulation in a multispecies diatom biofilm

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ABSTRACT

While the effects of abiotic parameters on microbial tidal biofilms are relatively well-documented, the effects of grazing and/or bioturbation by meiofauna are poorly understood. We investigated the impact of a natural nematode assemblage on the biomass and microbial community structure of a multispecies diatom biofilm. Nematodes stimulated diatom biomass accumulation of the biofilm and caused a shift in diatom community structure. Higher diatom biomass accumulation in the presence of nematodes could be the result of increased diatom biomass production through nutrient regeneration resulting from grazing or bioturbation, and/or through shifts in interspecific interactions between diatoms (e.g. competition) through selective grazing. Alternatively, lower biomass in the controls may be due to higher secretion of diatom production in the form of bound extracellular polymeric substances (EPS). Our observations underscore that meiobenthos, and especially nematodes, are important for the structure and production of tidal biofilms.

1. Introduction

Tidal flats are among the most productive habitats on earth and provide valuable ecological and economical services (MacIntyre et al., 1996; Underwood and Kromkamp, 1999). The surface layer of intertidal sediments can be covered by extensive and dense biofilms composed of benthic microalgae (microphytobenthos, MPB) and bacteria embedded in a matrix consisting of Extracellular Polymeric Substances (EPS) (Smith and Underwood, 1998). EPS is produced by diatoms, but also by bacteria (Gerbersdorf et al., 2009) and Archaea (Poli et al., 2011), for substrate attachment, locomotion, protection against desiccation and carbon overflow (Wotton, 2004). MPB, and in particular diatoms, constitute an important primary resource for benthic food webs in tidal flat and salt-marsh sediments (Degré et al., 2006; Middelburg et al., 2000; Moens et al., 2005a, 2002; Rzeznik-Orignac et al., 2008). Additionally, the secretion of EPS by MPB increases sediment stability (Gerbersdorf and Wieprecht, 2015; Ubertini et al., 2015) and as such also influences the occurrence and abundance of several macrofauna and meiofauna species (Van Colen et al., 2009).

MPB community structure and biomass are regulated by a wide range of abiotic and biotic factors (Van Colen et al., 2014). Abiotic drivers include irradiance, temperature, hydrodynamic disturbance by tides and weather (e.g. wind), substrate type (grain size) (Ubertini et al., 2015), salinity and nutrient availability (Barranguet et al., 1998; Paterson and Hagerthey, 2001; Ubertini et al., 2015), the relative importance of which varies in space and time. In addition to these abiotic drivers, various biotic factors influence the development of diatom biofilms, including grazing and/or bioturbation by benthic fauna (Orvain et al., 2004; Underwood and Kromkamp, 1999). Several studies have reported a decrease and even a collapse of MPB biofilms in the presence of macrobenthic grazing (Hagerthey et al., 2002; Hillebrand and Matthiessen, 2009; Pratt et al., 2015). The effects of macrofauna grazing are often difficult to predict as they arise from the complex interplay of various processes including grazing intensity and selectivity, prey resistance, and changing interactions within the microbial biofilm community (e.g. competitive and synergistic diatom interactions) (Hagerthey et al., 2002), and as such also depend on the density and (functional) diversity of both the microbial and the macrofauna community (Van Colen et al., 2015). Likewise, the effect of bioturbation is unpredictable. Bioturbation may increase solute transport rates, thereby enhancing nutrient resupply and stimulating MPB production (Berg et al., 2001; Braeckman et al., 2010). However, bioturbation can also enhance sediment erodibility and resuspension by increasing sediment water content and surface micro-topography (Orvain et al., 2004).

Whereas the effects of macrobenthic organisms on MPB have received considerable attention, the impact of meiofauna on the biomass and structure of intertidal biofilms is poorly known. Nematodes are

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usually by far the most abundant and species-rich metazoan meiofauna in soft sediments, particularly in estuarine intertidal areas (Soetaert et al., 1994), reaching densities $> 10^6\,m^{-2}$ and a local diversity of tens of species m^{-2} (Heip et al., 1985). A broad range of meiofauna species graze on diatoms (Hicks and Coull, 1983; Moens et al., 2014; Moens and Vincx, 1997) and as such contribute to the transfer of energy and matter in these systems, as they in turn form a food source for a variety of secondary consumers (Coull, 1999). Nevertheless, there is no consensus as to whether meiofaunal grazing in general, and nematode grazing in particular (Blanchard, 1991), affects growth rates, standing stocks and diversity of benthic diatom communities. Both pronounced (Blanchard, 1991; Montagna, 1984; Montagna et al., 1995), moderate (Rzeznik-Orignac and Fichet, 2012) and negligible (e.g. Middelburg et al., 2000) top-down effects of nematodes on MPB biomass have been reported. These estimates are in most cases based on short-term (hours to days) measurements of meiofaunal grazing rates, compared with measurements of MPB biomass or primary production (Montagna et al., 1995). It has also been hypothesized that nematodes can stimulate MPB growth, possibly through microbioturbation, the production of N-rich excreta and/or indirectly through bacteria-MPB interactions (Hubas et al., 2010). Meiofauna can stimulate the bacterial mineralization of organic matter (Nascimento et al., 2012; Rysgaard et al., 2000) and increase denitrification (Bonaglia et al., 2014; but see Stock et al., 2014), although they have also been shown to reduce mineralization rates in other studies (De Mesel et al., 2003; Näslund et al., 2010). Nematodes can also stimulate bacterial growth and EPS production (Hubas et al., 2010) and change the composition of bacterial assemblages (De Mesel et al., 2004). The underlying mechanisms may involve selective grazing on bacteria (e.g. De Mesel et al., 2004), the production of mucus tracks which offer a substratum for microbial attachment and growth (Mathieu et al., 2007; Moens et al., 2005b), the excretion of N-rich compounds (Gebremikael et al., 2014), or microbioturbation (Aller and Aller, 1992; Berg et al., 2001; Bonaglia et al., 2014). These mechanisms may also extend to nematode effects on microphytobenthic primary production and EPS secretion (Hubas et al., 2010; Moens et al., 2014).

In the present study, we examined the effect of a natural nematode community on the development and structure of a multispecies diatom biofilm. The experiment was carried out in modular microcosms for use in aquaria with a tidal regime, which allowed controlling and replicating the composition and biomass of both diatom and nematode communities in a tidal setting. We assessed the effect of nematodes on diatom biomass and community composition, bacterial community composition, EPS production and total organic carbon and total nitrogen content of the sediment.

2. Materials and methods

2.1. Microcosm design and experimental set-up

A microcosm was developed for use in aquaria with a tidal regime (Fig. 1a), allowing full control over diatom and nematode community composition and biomass. The modular microcosms were constructed from acrylic glass and the different components were melted together using chloroform. They had an inner diameter of 36 mm (creating an internal surface area of $\sim 10\,cm^2$) and a height of 10 cm. Three side apertures at a height of 15 mm allowed exchange of dissolved substances (incl. nutrients) during high tide. The apertures had an inner diameter of 16 mm and were sealed with glass fibre filters with a pore size close to 1.2 μm (Whatman® glass microfiber filters, Grade GF/C). At the bottom, the microcosms were sealed with a glass fibre filter (GF/C) and an additional nylon-woven monofilament fabric (Sefar®, pore size 10 μm) for structural support. The filter membranes at the bottom and the side apertures of the microcosms allowed complete drainage and flooding at realistic rates during tide simulation, while still enabling to separately control the diversity, composition and biomass of nematodes and MPB inside each microcosm, since cells > 1.2 μm cannot cross the membranes. To further avoid contamination by accidental 'splashing' of water droplets over the top of a microcosm, the top was covered with a nylon fabric (Sefar®, pore size 10 μm) that was held in place by sliding a ring over the microcosm (Fig. 1a). The simple modular design and small size of the microcosms allow for easy replication, manipulation and sampling of replicates and/or treatments. Moreover, variations on the design can easily be made, for instance to allow for a higher sediment column or surface area.

The experiment consisted of two treatments, each starting with 12 replicates: the control treatment (C) contained a multispecies diatom assemblage without nematodes, while the nematode treatment (N) consisted of the same diatom assemblage incubated together with a natural nematode assemblage. Five hundred nematodes (randomly picked from the pool of extracted nematodes, cf. below) were added to each of the 12 microcosms of the N treatment, resulting in microcosm densities reflecting intermediate *in situ* spring densities at the sampling site ($\sim$500 ind/10 cm^{-2}) (Bezerra, unpubl.). An additional 100 nematodes were randomly picked to assess the initial composition of the nematode assemblage. Diatoms and nematodes were incubated in the microcosms on 22 g of sandy sediment (median grain size 248 μm, no silt present). Sediment was collected at the Paulina intertidal flat (51°20′58.2″N 3°43′54.1″E, The Netherlands) from the top 1 cm layer. It was defaunated by sieving over a 1-mm mesh followed by drying at 110 °C during 48 h. After addition of the sediment to the microcosms, diatoms were introduced to each microcosm, followed by the transfer of the nematodes using the tip of a fine needle.

The microcosms were then incubated into four separate tidal aquaria, with three replicates of each treatment per aquarium. The aquaria (Fig. 1b) were equipped with two pump systems connected to a timer, which allowed simulating a tidal regime. The transition from high to low tide took approximately 20 min and was implemented by pumping water out of, and into the aquaria. At high tide, the water column extended 4 cm above the sediment surface whereas at low tide, the water level was 1 cm below the sediment surface. Both treatments were incubated for 16 days at a temperature of 15 °C in autoclaved seawater from the Paulina site (salinity of 24), enriched with Guillard F/10 medium + Si (Guillard and Ryther, 1962), which was refreshed at the start of each high tide. The aquaria were subjected to a daily 8-h photoperiod with a light intensity of 28 μmol photons $m^{-2}\,s^{-1}$ (produced by L58W/840 Lumilux cool white fluorescent tubes) and a 6-h low-tide period, starting 1.5 h after the onset of light (Fig. 1c).

2.2. Diatom culture

The experiment was carried out using a mixture of four different diatom species, viz. *Navicula arenaria, Navicula phyllepta, Amphora* sp. and *Seminavis robusta*, covering a range of cell sizes (Table 1) and shapes. All strains were obtained from the diatom culture collection (BCCM/DCG) of the Belgian Coordinated Collection of Micro-organisms (http://bccm.belspo.be/about-us/bccm-dcg) (DCG accession numbers: 0489, 0476, 0481 and 0105). The three former strains originate from the Paulina intertidal flat (Table 1) in the polyhaline part of the Scheldt estuary and represent common and abundant species in the biofilms at this site (which typically contain between 6 and 8 dominant diatom species (Forster et al., 2006)), and elsewhere (e.g. Sundbäck et al., 2000). The *S. robusta* strain is progeny of isolates collected from a littoral site in the Veerse Meer, a brackish-water lake in The Netherlands (51°32′36″N; 3°48′15″E), and was grazed upon by several of the more abundant nematode species from the Paulina tidal flat in microcosm experiments (Moens et al., 2014). All species were cultured separately in natural seawater supplemented with silicate enriched Guillard F/2 medium (Guillard and Ryther, 1962) and incubated at 18 °C with a 12 h light: 12 h dark photoperiod (light intensity E, 23 μmol $m^{-2}\,m^{-1}$, produced by L58W/840 Lumilux cool white fluorescent lamps). All four species were combined just before inoculation and this mixture was