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SHORT COMMUNICATION

Seasonal microbial community dynamics correlate with phytoplankton-derived polysaccharides in surface coastal waters

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Phytoplankton produce large amounts of polysaccharide gel material known as transparent exopolymer particles (TEP). We investigated the potential links between phytoplankton-derived TEP and microbial community structure in the sea surface microlayer and underlying water at the English Channel time-series station L4 during a spring diatom bloom, and in two adjacent estuaries. Major changes in bacterioneuston and bacterioplankton community structure occurred after the peak of the spring bloom at L4, and coincided with the significant decline of microlayer and water column TEP. Increased abundance of *Flavobacteriales* and *Rhodobacterales* in bacterioneuston and bacterioplankton communities at L4 was significantly related to the TEP decline, indicating that both taxa could be responsible. The results suggest that TEP is an important factor in determining microbial diversity in coastal waters, and that TEP utilisation could be a niche occupied by *Flavobacteriales* and *Rhodobacterales*.
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Bacterioplankton communities at the English Channel L4 time-series station have repeating seasonal-scale diversity patterns (Gilbert *et al.,* 2012). *Rickettsiales* dominate during the winter when primary production is low, whereas *Rhodobacterales* dominate during the spring and autumn when primary production is high (Gilbert *et al.,* 2009, 2012). Similar changes in diversity during and following spring diatom blooms in the North Sea indicate that specialised bacterioplankton populations are adapted to utilise phytoplankton-derived organic matter (Teeling *et al.,* 2012). Bacterioplankton carbohydrate-active enzymes (CAZymes) increase with phytoplankton blooms, suggesting that phytoplankton-derived polysaccharides are particularly important in affecting bacterioplankton diversity (Teeling *et al.,* 2012).

Transparent exopolymer particles (TEP) are polysaccharide gels that are formed from phytoplankton-derived precursors, are abundant in seawater and typically peak in concentration towards the end of phytoplankton blooms (Passow, 2002). TEP from the water column can rise up to the air–sea interface (sea surface microlayer; Azetsu-Scott and Passow, 2004), and form directly in the microlayer (Wurl *et al.,* 2011). TEP in the sea surface microlayer are important because they contribute to the physicochemical structure of the microlayer, and could provide habitat and resource for microlayer bacteria (bacterioneuston; Cunliffe *et al.,* 2013).

This study aimed to determine potential links between the bacterioplankton and bacterioneuston communities at L4 station with TEP. We also incorporated samples collected from two estuaries in close proximity to L4 in order to establish a broader geographical perspective (Figure 1a). 16S rRNA genes were amplified from extracted DNA using primers 515F and 806R (Caporaso *et al.,* 2011), sequenced using the Ion Torrent PGM and analysed with the QIIME software package (Caporaso *et al.,* 2010; Supplementary Materials and Methods).

Comparison between L4 operational taxonomic unit (OTUs) showed that bacterioplankton and bacterioneuston communities changed considerably following the peak of the spring diatom bloom (Figures 1b and c, Supplementary Figure 1). Based on the presence/absence of OTUs, estuarine bacterioplankton and bacterioneuston communities were distinct from the L4 communities (Figure 1b), however, some estuarine communities became similar to the L4 post-phytoplankton bloom communities when the relative abundance of OTUs was considered (Figure 1c).

Previous studies have raised the issue of the representativeness of L4 station (Caporaso *et al.,* 2012; Gilbert *et al.,* 2012). This study indicates that

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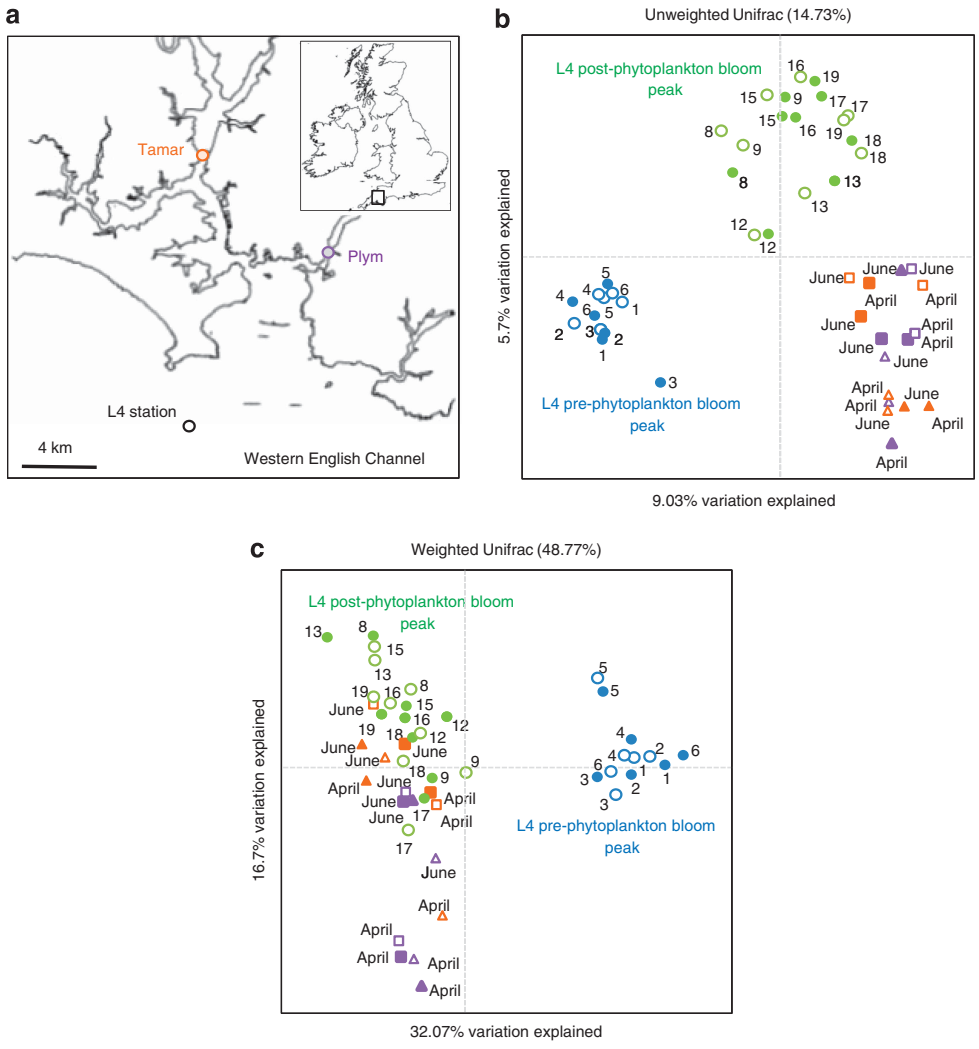

Figure 1 (**a**) Location of L4 station in the Western English Channel and estuarine sampling sites on the Tamar and Plym estuaries. (**b**) Principal coordinates analysis plot describing betadiversity using the unweighted UniFrac distance matrix generated from OTU (97% similarity) presence/absence. Fifteen surveys were conducted over 19 weeks between 9 February and 2 July 2012 (numbers indicate week surveyed). Microlayer-enriched samples were collected using a mesh screen (sampling depth $\leqslant$400 μm; open shapes) and underlying water samples were collected from 2 m (closed shapes). Estuarine microlayers and underlying water were sampled twice during the study period; 25 April and 27 June Tamar high tide (orange squares) and Tamar low tide (orange triangles), and 26 April and 29 June Plym high tide (purple squares) and Plym low tide (purple triangles). (**c**) The same analysis as **b** except using a weighted UniFrac distance matrix generated from OTU relative abundance.

the L4 station community during periods of high primary productivity is similar to the communities from neighbouring estuarine ecosystems.

In parallel with microbial community analysis, TEP were quantified using the alcian blue dye-binding assay (Passow and Alldredge, 1995). L4 microlayer and underlying water TEP concentrations were coupled throughout the study (Spearman Correlation $r=0.77$, $P<0.01$, $n=16$), increasing with the spring bloom up to week 3, and rapidly decreasing between weeks 7 and 9 (Figure 2a). The same pattern of increase and rapid decrease in TEP concentration also occurred during the secondary phytoplankton bloom. TEP was significantly lower (*t*-test; $P<0.01$) in both the microlayer and underlying water after the spring bloom peak when the microbial communities had changed. The decline in TEP was not related to changes in phytoplankton biomass (chlorophyll-*a*) at L4. Average estuarine TEP concentrations were 799 ± 210 μg carbon per l and 954 ± 237 μg carbon per l in the subsurface and microlayer, respectively, and were significantly higher than the TEP concentrations at L4 during the same period (*t*-test; $P<0.01$).

We compared changes in TEP concentration (Figure 2a) with microbial community composition (Supplementary Figure 2) to identify potential groups linked to the rapid decreases in TEP at L4. The orders *Flavobacteriales* and *Rhodobacterales* in the subsurface and microlayer communities were negatively correlated with TEP concentration (*Flavobacteriales,* Spearman Correlation $r=-0.50$, $P<0.01$, $n=28$; *Rhodobacterales,* Spearman Correlation $r=-0.55$, $P<0.01$, $n=28$), both significantly

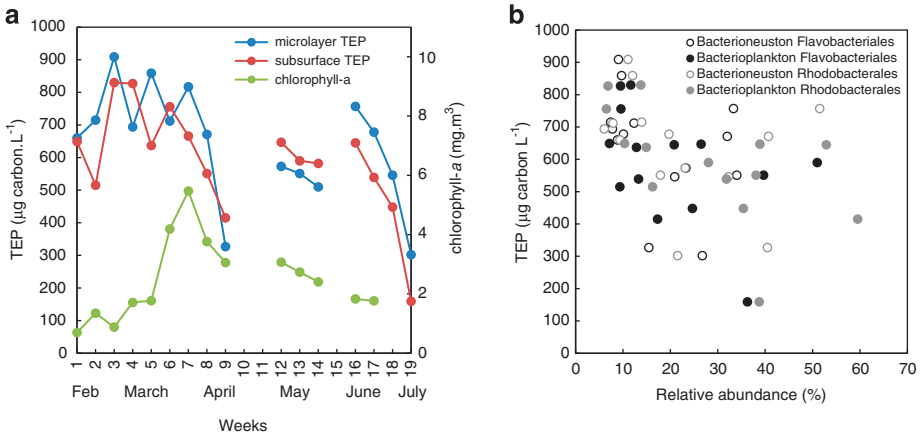

Figure 2 (**a**) Transparent exopolymer particle (TEP) concentration in the microlayer and underlying water at L4 station. Surveys were conducted over 19 weeks between 9 February and 2 July 2012. Phytoplankton biomass at L4 station was determined from chlorophyll-*a* concentration in underlying water samples. (**b**) Relationship between the increase in abundance of the orders *Flavobacteriales* and *Rhodobacterales* in both bacterioneuston and bacterioplankton communities and the decline of TEP in the microlayer and underlying water at L4 station.

increasing in abundance when TEP was depleted (Figure 2b). No components of the estuarine microbial communities showed similar links to TEP.

Genome analysis of *Flavobacteriales* isolates has identified high numbers of genes encoding the CAZymes glycoside hydrolases (Fernández-Gómez *et al.,* 2013), and a mesocosm study has shown that extracellular glycoside hydrolases degrade diatom TEP (Smith *et al.,* 1995). TEP are sulfated polysaccharides that are enriched with fucose (Passow, 2002), and *Flavobacteriales* sulfatases and fucosidases increase with spring diatom blooms (Teeling *et al.,* 2012). Combined with the evidence from our study, we propose that the *Flavobacteriales* at L4 are degrading and utilising phytoplankton-derived TEP.

The *Rhodobacterales* at L4 were primarily members of the Marine Roseobacter Clade (MRC). The MRC are a metabolically diverse and opportunistic bacterioplankton group (Newton *et al.,* 2010), which are known to form close associations with phytoplankton blooms (Geng and Belas, 2010). MRC responding to diatom blooms in the German Bight increase expression of low-molecular-weight substrate transporters (Teeling *et al.,* 2012). It is possible that the *Rhodobacterales* at L4 could be utilising low-molecular-weight substrates produced from the *Flavobacteriales*-degraded TEP via cross-feeding. Supporting this idea, Gilbert *et al.* (2012) showed that bacteria–bacteria interactions are very important at L4 station.

Caporaso *et al.* (2012) have proposed that L4 bacterioplankton diversity is a result of induced changes in the abundances of a persistent and resident community. Here we show that phytoplankton-derived TEP could contribute to some of the induced community diversity responses at L4, namely, the seasonal increase of the orders *Flavobacteriales* and *Rhodobacterales*.

There is a need to connect seasonal changes in microbial community composition with geochemical processes (Giovannoni and Vergin, 2012). Marine gel particles are released from the sea into the atmosphere by fragmentation of the sea surface microlayer during bubble bursting (Russell *et al.,* 2010), and are subsequently a source of aerosols and cloud condensation nuclei (Orellana *et al.,* 2011). The realisation of the importance of marine gels in climate regulation has forced a fundamental paradigm shift away from an exclusively dimethylsulfide-controlled marine boundary layer, to a complex system where marine gels are integral (Quinn and Bates, 2011). This study is the first to link changes in microbial diversity with marine gels, and therefore supports a multidisciplinary understanding of marine gel biogeochemical cycling. The coupling between microlayer and underlying water TEP concentrations and bacteria community dynamics indicate that microbial TEP processing could be similar in both systems.

Conflict of Interest

The authors declare no conflict of interest.

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Supplementary Information accompanies this paper on The ISME Journal website (http://www.nature.com/ismej)