Microalgal primary producers and their limiting resources
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CHAPTER 5

Absence of microphytobenthos suspension in the western Dutch Wadden Sea: benthic and pelagic community analyses

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In preparation

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Abstract

Benthic and pelagic primary producer communities have been investigated at different sampling seasons (April, May/June and September in 2011) across three pelagic stations (S18, S19 and S20) and two benthic stations (TfS20 and TfS21) by comparing two methods: a molecular fingerprint, denaturing gradient gel electrophoresis (DGGE) and a chemotaxonomic biomarker, phospholipid fatty acid (PLFA). Results from cluster analyses showed that both methods were able to distinguish spatial and temporal changes in benthic and pelagic primary producers. The DGGE cluster analysis showed a clear separation between the benthic and pelagic communities whereas the PLFA cluster analysis showed overlap in the benthic and pelagic communities during May/June. This apparent discrepancy between the two methods may be attributed to different taxonomic resolution and physiological status of the studied communities. Hence, although the mesotidal system of the Marsdiep basin is strongly affected by wind and tidal forces, the data suggested that no major suspension events of MPB were observed. Pelagic cyanobacterial and eukaryotic communities showed a clear seasonal pattern, but no strong seasonal signal was observed in the benthic communities. Influence of freshwater discharge from Lake IJsselmeer may have influenced the cyanobacterial community at S18, which seemed to influence the cyanobacterial community more than the eukaryotic community. Although the C-fixation in MPB can exceed the C-fixation in phytoplankton (in September), the overall contribution of the MPB primary production to the annual aquatic primary production in the intertidal flats was lower than the phytoplankton primary production in the Marsdiep basin of the western Dutch Wadden Sea.
**Introduction**

In intertidal ecosystems, microphytobenthos (MPB) is an important driver of the biogeochemical cycles in the sediment (Paerl & Pinckney, 1996). Primary production of MPB can reach up to 50% in these ecosystems (Underwood & Kromkamp, 1999). Bed sediment in tidal basins consists of a mixture of sandy and muddy sediments (van Ledden et al., 2004). The muddy sediments are mainly composed of silt and clay (particle size <63 µm), an environment rich of organic matter that can host epipelic diatom community. In this type of sediment, MPB plays a role in sediment cohesion and stabilization (De Brouwer et al., 2000). In the transition of muddy to sandy environment, sediment displays less cohesiveness (Winterwerp & van Kesteren, 2004). An environment with coarse sand supports an epipsammic community, i.e. cells attached to the grains with limited motility (MacIntyre et al., 1996, Consalvey et al., 2004, Jesus et al., 2009). The sediment properties are partly a reflection of the hydrodynamic energy in the system and therefore govern MPB composition (Jesus et al., 2009, van der Wal et al., 2010). In addition, seasonal patterns of biological processes originate from higher trophic level may disrupt the stability of the bed sediment by modulating MPB biomass in the sediment with grazing or bird feeding activity (Daborn et al., 1993), making it more prone to erosion. Unlike the clear seasonality observed in phytoplankton blooms of temperate estuarine and coastal systems (Winder & Cloern, 2010), recurrent seasonality patterns of MPB community are more difficult to predict due to a combination of episodic abiotic events such as strong winds that affect erosion and deposition (De Jonge, 1985, Montani et al., 2003, Kang et al., 2006, Ubertini et al., 2012). As a consequence, MPB biomass and species composition can be reset episodically during the year (De Jonge, 1985, Ubertini et al., 2012).

In the eastern Wadden Sea (Ems estuary) where a muddy environment is dominant, up to 30% of the MPB biomass was found in the water column (De Jonge, 1985, De Jonge & Van Beusekom, 1992). Suspension of MPB cells increases pelagic primary production and sediment suspension may alleviate nutrient limitation in the water column (Boero et al., 1996, Coma et al., 2000, Tengberg et al., 2003, Forehead et al., 2013, Leote et al., submitted). Erosion of tidal flats can influence the concentration and composition of organic matter, nutrient fluxes and metabolic activity of the benthic community (Forehead et al., 2013). On the opposite, calm conditions will favor intact MPB biofilms which can act as a trap for nutrients released from the sediment. In the Wadden Sea tidal flats the rate of MPB photosynthesis and nutrient fluxes are variable and depend on the type of sediment (Billerbeck et al., 2007).

Thus far, little work has been done on the exchange between communities of benthic and pelagic primary producers in response to environmental change in the area of the western Dutch Wadden Sea. To fill up these gaps, we characterized benthic and pelagic primary producer communities at different temporal-spatial scales by applying two methodological approaches: phospholipid fatty acid (PLFA) composition of phytoplankton and MPB and denaturing gradient gel electrophoresis (DGGE) targeting the cyanobacterial 16S rRNA- and the micro-Eukarya 18S rRNA genes. PLFA are mainly associated with cell membranes and have short life times after their release in the environment. Thus, PLFA can be used as indicator of actual living biomass.
and as a chemotaxonomic marker because their composition differs between taxonomic groups (Vestal & White, 1989, Dalsgaard et al., 2003, Bianchi & Canuel, 2011, Dijkman et al., 2009). In addition, we also used DGGE to discriminate between benthic and pelagic microorganisms. We investigated the benthic and pelagic primary producer communities at different pelagic and intertidal stations and at three different stages during their seasonal growth cycle in the Marsdiep basin of the western Dutch Wadden Sea in order (1) to investigate whether MPB is suspended into the water column by using two methods, PLFA and DGGE, (2) to estimate MPB primary production and compare it with pelagic primary production.

**Material and methods**

*Study sites*- Seawater was collected at three pelagic stations (S18, S19 and S20) during three sampling periods in 2011 from: Navicula 7 (Nav7: 18<sup>th</sup>-21<sup>st</sup> of April), Navicula 8 (Nav8: 30<sup>th</sup> of May-1<sup>st</sup> of June) and Navicula 9 (Nav9: 26<sup>th</sup>-29<sup>th</sup> September) with the R/V Navicula during high tide (Fig. 1). The coordinates for each station are shown in Table 1. Additionally, MPB communities from the tidal flat were sampled at two different locations TfS20 and TfS21 during low tide. The pelagic locations were chosen in such a way that they gave easily access to the nearby tidal flats. A third station TfS18 was also sampled, but the MPB biomass on this sandy tidal flat was so small causing large uncertainties in the data, that we did not include the data in the results.

*PLFA extraction from the water column and sediment*

PLFA from the water column were sampled using a Niskin bottle just below the surface and filtered onto pre-combusted glass-fiber filters (Whatman GF/F). PLFA from the tidal flats were collected by scraping the top centimeters from the sediment. All samples were kept at -80 °C until analysis. PLFA samples were extracted using a modified Bligh and Dyer method (1959) (Middelburg et al., 2000). Lipids were extracted in a mixture of chloroform:methanol:water (1:2:0.8, v:v:v). The extraction fluid was evaporated by shaking for at least two hours at speed 190 rpm using a vacuum extractor (Rapid Vap®, Labconco Corp., Kansas City, MO, USA). The formation of an aqueous-organic two layer system was induced by the addition of chloroform and water ratio of chloroform:methanol:water (1:1:0.9, v:v:v). The lower phase of chloroform containing the total lipid extract was collected. After evaporation of the solvent, the total lipid extract was fractionated into different polarity classes on silica columns (0.5 g Kieselgel 60; Merck) and eluted sequentially with chloroform:acetone:methanol (1:1:2, v:v:v). The methanol fraction containing the PLFA was collected. After evaporation of the methanol, a mixture of methanol:toluene was added (1:1, v:v) and methyl ester derivatives of the fatty acids (FAME) were synthesized using mild alkaline methanolysis (1 mL of 0.2 mol L<sup>-1</sup> of sodium methylate). In order to stop the methylation reaction, a mixture of hexane:acid acetic:milliQ (1:0.3:1, v:v:v) was added. The upper layer of the aqueous-organic phase separation containing hexane was collected. In addition, 20 µL of each internal standard (19:0 and 12:0, both 0.1 mg) was added during the synthesis of the derivatives. The carbon isotopic composition of each individual
FAME was determined with GC-C-IRMS, using a Varian 3400 gas chromatograph equipped with a Varian SPI injector, which was coupled via a type II combustion interface to a Finnigan Delta S isotope ratio mass spectrometer (Middelburg et al., 2000). The FAMEs were identified according to their retention times compared to a reference standard.

**Extraction of nucleic acids**

Samples for DNA extraction were taken from the water column at high tide and 0.5 mg sediment during low tide. For the water column samples, 200-250 mL samples were filtered over 0.2 µm polycarbonate filters and stored at -80°C until use. Filters were cut into small pieces (area of 0.3 mm²) using a sterile scalpel and DNA was extracted using the UltraClean Soil DNA isolation kit (Mo Bio Laboratories, Inc.) according to the manufacture’s recommendation. The same kit was used for extraction of DNA from sediment samples. DNA concentration and purity were determined with a spectrophotometer NanoDrop ND 1000 (NanoDrop Technologies, Inc., Wilmington, DE 19810, USA).

**PCR amplification of 16S- and 18S rRNA genes**

To amplify the 18S rRNA-Eukarya gene, we used primer pair A (EK1-F and EK1520R) that amplifies a 1520 bp long fragment and primer pair B (EK1-F and EUK 516r-GC) that amplifies a 556 bp long fragment, and which includes the GC-clamp necessary for DGGE. The 16S rRNA gene fragments from cyanobacteria were amplified using primer pair A (CYA 359f, U1492R) that amplifies 1520 bp long fragment and primer pair B (CYA 359f-GC and CYA 781R a/b) that amplifies a 462 bp long fragment. The sequences of the primers used are shown in Table 2.

For the DGGE analysis, a nested PCR was performed. In the first PCR, 16S- and 18S-rRNA genes amplification was performed using primer pairs A followed by a second PCR reactions using primer pairs B. The first PCR was performed in a 25 µL volume containing 2µL of DNA template (final concentration 200 ng), 2.5 µL of 10 × PCR buffer (New England Biolabs), 0.125 µL of DMSO (3% final concentration), 2.5 µL of 0.1% w/v bovine serum albumin (BSA), 0.5 µL dNTP (10 mM), 0.5 µL of each primer (10 µM), 0.125 µL of Taq DNA polymerase (New England Biolabs) (2 U/µL) and 15.125 µL of diethyl pyrocarbonate-treated water (DEPC). The second PCR was performed in a volume of 25 µL containing 2 µL of PCR products from the first amplification, 2.5µL of 10 × PCR buffer (GE Healthcare), 1.25 µL of DMSO, 2.5 µL of BSA (0.1% w/v), 0.5 µL of dNTP (10mM), 0.5 µL of each primer, 15.125 µL of DEPC, 0.125 µL of 1 U/µL rTaq DNA polymerase (GE Healthcare). Between the first and the second PCR, the PCR products were purified with a Sephadex G-50 Superfine kit (Sigma-Aldrich) to remove primers and other PCR components that may interfere with the second PCR. The PCR cycling conditions are described in Table 3.
**DGGE analysis**

PCR products were separated based on their GC contents on a denaturing gel containing a gradient of the denaturants urea and formamide ranging from 10% to 60% for 16S rRNA, and from 30% to 55% for the 18S rRNA. Electrophoresis was performed at 60 °C and 100 V during 16 hours on the PhorU DGGE system (Ingeny, Goes, Netherlands). After electrophoresis, the gel was stained with silver nitrate according to the protocol of Bolhuis et al. (2013).

**13C incubation procedures**

Water samples were collected with a Niskin bottle just below the water surface. Samples were divided into four polycarbonate bottles of 10L each. Water samples were enriched with NaH\textsubscript{13}CO\textsubscript{3} (99% \textsuperscript{13}C; Cambridge Isotope Laboratories, Inc.) to 4% of the ambient dissolved inorganic carbon (DIC) (2.2 mmol L\textsuperscript{-1}, E. Epping, pers. communication) and incubated under artificial light at 100 µmol photons m\textsuperscript{-2} s\textsuperscript{-1} for two hours. POC samples were separately filtered before and after \textsuperscript{13}C incubation onto pre-combusted glass fiber filters (Whatman GF/F). The \textsuperscript{13}C stable isotope composition of the samples was analyzed with a Carlo Erba elemental analyzer coupled inline with a Finnigan Delta S isotope ratio mass spectrometer (EA-IRMS). Excess \textsuperscript{13}C (µg L\textsuperscript{-1}) in POC was calculated following isotopic calculations according to Middelburg et al. (2000). Stable isotope data are expressed in the delta notation (δ\textsuperscript{13}C) relative to carbon isotope ratio (R = \textsuperscript{13}C/\textsuperscript{12}C) of Vienna Pee Dee Belemnite (R\textsubscript{VPDB} = 0.0112372): δ\textsuperscript{13}C = [(R\textsubscript{sample}/ R\textsubscript{VPDB}) - 1 × 1000]. The \textsuperscript{13}C fixation into bulk organic matter (µg C L\textsuperscript{-1} h\textsuperscript{-1}) was calculated from the difference of the fraction of \textsuperscript{13}C in POC at the start and at the end of the incubation, multiplied by the POC concentration at the start of the incubation. The fraction of \textsuperscript{13}C in POC was calculated as \textsuperscript{13}C/ (\textsuperscript{13}C + \textsuperscript{12}C) = R/ (R+1). In the water column, \textsuperscript{13}C fixation into bulk organic matter (mg C m\textsuperscript{-2} h\textsuperscript{-1}) was calculated per unit surface by integrating production rates over the water depth (z = 4.5 m). For each tidal flat station, the \textsuperscript{13}C labeling experiment was performed using a frame of 0.25 m\textsuperscript{2} during the emersion period. A volume of 250 mL containing \textsuperscript{13}C labeled bicarbonate (NaH\textsubscript{13}CO\textsubscript{3}) solution was sprayed to the entire sediment surface delimited by the frame. The NaH\textsubscript{13}CO\textsubscript{3} added to the sediment represented 40% of the DIC concentration in the overlying water, assuming that the added DI\textsuperscript{13}C was distributed in the top 3 mm of the sediment. POC samples were collected by scraping the top centimeter of the sediment before and after \textsuperscript{13}C incubation. The incubation was four hours, depending on the site and started within 30 min after the tide receded. Samples were kept at -80 °C until analysis. The \textsuperscript{13}C fixation into bulk organic matter (mg C m\textsuperscript{-2} h\textsuperscript{-1}) was calculated using a specific density of the sediment (σ= 2.65 kg dm\textsuperscript{-3}) (Slomp et al., 1997).

**Data analysis**

Cyanobacterial and eukaryal community analyses based on DGGE band patterns were analyzed using BioNumerics 6.6 software (Applied Maths, NV, Sint-Martens-Latem, Belgium). The DGGE banding patterns were examined in two ways, with a cluster analysis and with a Shannon’s diversity index analysis. A DGGE band was considered to represent an operational
taxonomic unit (OTU). The intensity of the DGGE band was taken as a measure for the relative abundance of this OTU in the community. From the number of OTU, the Shannon’s diversity index (H) was calculated (Shannon & Weaver, 1948). Using the number of bands, a matrix of similarity was obtained by applying the Jaccard algorithm. Based on this similarity matrix, unweighted pair-group averaging, the so-called UPGMA clustering method was applied using software Primer (version 6.1.12) (Clarke & Gorley, 2006). A similar clustering method was performed on the PLFA data (composition expressed as the mol percentage of total PLFA). Results from DGGE and PLFA are presented as dendograms. To test for significant differences (p<0.05) between stations and sampling periods in C-fixation rates in pelagic and benthic systems, two way analysis of variance (two-way ANOVA) was applied to the data.

**Results**

Fig. 2 shows three cluster analyses of DGGE band patterns with cyanobacterial 16S rRNA, eukaryal 18S rRNA and PLFA from five locations (three pelagic locations: S18, S19 and S20 and two benthic locations: TfS20 and TfS21).

**Cyanobacterial DGGE band patterns and diversity**

Cluster analysis of the cyanobacterial DGGE band patterns revealed two distinct groups which separated benthic (A1) and pelagic (A2) communities (Fig. 2A). None of the fingerprints within the two groups showed 100% similarity. Within the benthic group (A1), DGGE band patterns did not show apparent temporal and spatial grouping. On the opposite, the pelagic cyanobacterial group (A2) was divided into three subgroups, separating the three sampling periods (Nav7, Nav8 and Nav9). S18 did not group with the other stations during the Nav8 and Nav9 cruises. The cyanobacterial community at S18 from the Nav7 grouped with the Nav8 communities (with ~30% similarity), whereas the community from S18/Nav9 clustered more closely related to the communities found during Nav7 (~40% similarity). The cyanobacterial H-index in the pelagic stations was lower during Nav7 than during Nav8 and Nav9 (Fig. 3A). Diversity in the cyanobacterial benthic community was higher than in the pelagic communities during Nav7 and Nav9, whereas the H-index was comparable between both types of communities during Nav8. The highest H-index was observed at Tf20 (Nav9) (1.9) and the lowest value was observed at TfS21 (Nav7) (0.7).

**Eukaryal DGGE band patterns and diversity**

Cluster analysis of the eukaryal DGGE band patterns revealed a higher number of bands than for the cyanobacterial DGGE (Fig. 2B). Similar to the cyanobacterial cluster analysis, eukaryal community cluster patterns revealed a clear distinction between benthic (B1) and pelagic (B2) communities. The eukaryal benthic cluster B1 revealed two major subgroups. One subgroup comprised the communities of Tfs21 during Nav7 and Nav8. Only during Nav9, both stations Tfs20 and Tfs21 formed one group. Clustering analysis of the eukaryal pelagic DGGE
bands showed a clear temporal pattern (B2): each of the three seasons formed a separate cluster with a similarity of approximately 55%. Within each of these clusters, S18 and S19 were more similar to each other than to S20 during Nav8 and Nav9, whereas during Nav7, S18 was more similar to S20 than to S19. The H-index in eukaryal communities was higher than for the cyanobacterial communities (Fig. 3B). The H-index for the eukaryal pelagic community only showed little variability between the stations and the three different seasons. The eukaryal benthic H-index at the two stations was more variable although no particular pattern was discerned. At TfS20, diversity was lower than at TfS21 during Nav7 and the situation was opposite during Nav8. The H-index were similar between the two benthic communities (TfS20 and TfS21) during Nav9. Overall, eukaryal diversity was higher in the pelagic than in the benthic communities.

**PLFA composition pattern**

A cluster analysis based on the relative abundance of PLFA is depicted in Fig. 2, C. The cluster analysis included PLFA synthesized by bacteria (branched fatty acids), but removing these branched fatty acids from the analysis did not affect the clustering patterns. The cluster analysis without bacterial PLFA revealed two groups. Cluster C1 included all the benthic and pelagic stations sampled during Nav8, and they overlapped with a similarity close to 75%. The pelagic stations sampled during Nav7 and Nav9 clustered with a similarity around 70% (cluster C2), although S19 sampled during Nav7 did not group with the other two stations.

**C-fixation in phytoplankton and microphytobenthos**

The C-fixation rates of the phytoplankton community were significantly different between sampling periods (two-way ANOVA, \( p = 1.05 \times 10^{-6} \)) (Fig. 4). During Nav7, the C-fixation rates at S18 reached 18.2 mg C m\(^{-2}\) h\(^{-1}\) and were more than four times higher compared to stations S19 and S20 (two-way ANOVA, \( p = 3.18 \times 10^{-5} \)). Subsequently, during Nav8 and Nav9, the C-fixation rates decreased and values remained below 1.5 mg C m\(^{-2}\) h\(^{-1}\) at all stations. Although the C-fixation in MPB also showed significant differences between the sampling periods (two-way ANOVA, \( p = 1.1 \times 10^{-6} \)), the C-fixation rates in MPB followed a different trend compared to the C-fixation in the phytoplankton community. During the three sampling periods, the C-fixation rates were below 3.2 mg C m\(^{-2}\) h\(^{-1}\) at all stations with the exception of TfS20 during Nav9 when the value reached 7.7 mg C m\(^{-2}\) h\(^{-1}\). During Nav8 and Nav9, stations TfS20 showed significantly higher values compared to TfS21 (two-way ANOVA, \( p = 9.8 \times 10^{-8} \)).
Discussion

This study investigated spatial and temporal distribution of pelagic and benthic communities in the Marsdiep basin using two methodological approaches: DGGE and PLFA analyses. PLFAs are a good indicator of viable biomass but the taxonomic resolution is quite low (Dijkman et al., 2009, Bianchi & Canuel, 2011). The DGGE method has a higher taxonomic resolution; however, this method has its limitations. Because of its sensitivity, one species may display multiple bands on a DGGE gel and be erroneously interpreted as multiple species (Nübel et al., 1997, Muyzer, 1999, Díez et al., 2001). This study shows that molecular fingerprint and chemotaxonomic biomarker methods lead to partly different conclusions. However, the combination of both methods simultaneously provides information which cannot be obtained by either of them alone, and we will discuss them below.

No suspension of benthic communities occurs in the water column

Cluster analysis of the DGGE banding patterns showed that both cyanobacterial and eukaryal benthic communities were distinctively separated from the pelagic communities during all sampling periods. This indicates that mixing of pelagic and benthic compartments, either by sedimentation of the pelagic community or by suspension of the benthic community, was not important. As the hydrodynamic energy in the Marsdiep area is largely attributed to tidal and wind forces, sinking of the phytoplankton community is not likely to happen.

The results from the cluster analysis of the PLFA composition showed discrepancies with the DGGE fingerprints. The cluster analysis of PLFA data revealed an overlap between the benthic and the pelagic communities during the Nav8 cruise. The most likely explanation for this discrepancy with the DGGE results is that during the Nav8 sampling, the algal groups in the benthic and pelagic compartments were similar. At a higher taxonomic level, the PLFA composition between pelagic and benthic groups is not different (Dijkman et al., 2009, Dijkman et al., 2010, Kelly & Scheibling, 2012). An alternative to explain the overlap between benthic and pelagic communities during Nav8 could be that PLFA composition indicates similar physiological status between organisms (Dalsgaard et al., 2003, Piepho et al., 2012). The results of PLFA which agree only partly with the DGGE analysis, highlight the different sensitivities of the two methods. These findings disagree with results obtained by De Jonge & Van Beusekom (1992) who found that MPB in the eastern Dutch Wadden Sea contribute up to 30% of phytoplankton biomass. Hence, suspension of benthic microalgae may not be the major factor that increase the turbidity of the water and disrupt the phytoplankton primary production during these sampling periods (Schallenberg & Burns, 2004, MacIntyre et al., 2004, Porter et al., 2010). Minor seasonality in the benthic community- Phytoplankton communities in temperate regions often show a clear seasonal pattern whereas MPB seasonality is less conspicuous (Thornton et al., 2002, Winder & Cloern, 2010). Some studies show that MPB bloom reaches its maximum biomass between spring and summer (Montani et al., 2003, Ubertini et al., 2012) while other studies did not reveal any MPB seasonality (Thornton et al., 2002). We only sampled three
different periods during the algal growth season, our data lacked a clear seasonal signal in the MPB community. Only few studies investigated MPB seasonality in the Wadden Sea. The study by De Jonge & van Beusekom (1995) in the Ems estuary suggested that only one large bloom in MPB biomass occurs in summer between May and June. In the eastern part of the Wadden Sea (Ems Dollard) the mud content is often higher than in the tidal flats of the Marsdiep basin. The sampled stations are characterized as fine sand environment as the median grain size sediment $D_{50}$ values ranged between 158-213 µm (K. Philippart, pers. communication). A satellite based study on several estuaries bordering the North Sea demonstrated that MPB biomass changes in sandy locations are less affected by seasonality than MPB biomass occurring at muddy sites, which corroborates with our results (van der Wal et al., 2010).

Temporal and spatial patterns in pelagic and benthic communities

The eukaryal pelagic community revealed a distinct grouping between the different sampling periods, which is taken as an indication of a seasonal pattern. In contrast to the patterns observed for the eukaryotic pelagic communities, the cluster analysis showed that the cyanobacterial pelagic community depicts a less predictable seasonality as S18 did not cluster with other stations during Nav7 and Nav9. Less seasonality of the cyanobacterial community might be due to the fact that these organisms are present most of the year in the water column (Riegman et al., 1993). Furthermore, the pelagic cyanobacterial diversity during April (Nav7) was lower than in the other sampling periods. In contrast to pelagic cyanobacterial diversity, pelagic eukaryal diversity did not show particular trends. The decrease in cyanobacterial diversity might be attributed to a P limitation as the results obtained in April showed that autotrophic community experienced a P limitation (Ly et al., submitted). The cyanobacterial pelagic community and diversity may also be influenced by freshwater discharge from Lake IJsselmeer. Hence, S18 differed from the other stations because this station is located close to the locks of the Afsluitdijk, which separates Lake IJsselmeer from the Wadden Sea. This suggests that the freshwater discharge from Lake IJsselmeer influences S18 more than the other stations. The evidence that stations close to the Afsluitdijk have different properties was also observed from a PCA analysis carried out in chapter 2 where an analysis of physicochemical parameters and of PLFA composition and abundance showed that the station closest to the Afsluitdijk was separated from the other stations that form a tight cluster.

C-fixation rates in the pelagic and benthic communities of the Marsdiep basin

A comparison between the C-fixation of the benthic and pelagic communities (based on the $^{13}$C labeling in POC) has to be calculated with care. First uncertainty is that the total concentration of the $^{13}$C added is estimated assuming that the $^{13}$C is distributed homogenously in the top 3 mm of the sediment. The second uncertainty is that the DIC concentration in the upper lit layer of the sediment is not known. Because the incubations started immediately after the tide emerged, we assumed that the DIC concentration in the pore water was similar as in the seawater. However, photosynthesis will decrease the dissolved CO$_2$ concentration, but according to a modeling study by F. Meysman (pers. communication), the
bicarbonate concentration in the upper layers of the sediment stays more or less constant (in the absence of large bioturbators). Since the bulk of the DIC is bicarbonate and because most algae possess a carbon concentrating mechanism, it is likely that the total DIC concentration in the photic layer of the sediment will see only little change. It should be taken into account that the MPB derived C-fixation rates are the result of the activity of the whole photic zone, hence the average irradiance will be lower than the incident irradiance. When taking these uncertainties into account, the C-fixation rates of the pelagic and benthic microalgae can be compared. The results suggest that based on surface area the phytoplankton primary production exceeded the MPB production during spring (Nav7), but that at the end of the growth season in fall the MPB primary production was higher than the pelagic primary production (Nav9). At the end of spring (Nav8), the areal rates of primary production were similar. In order to extrapolate our data of the integrated primary production per hour to annual productions rates, we multiplied the obtained hourly rates times the number of days for one year and multiplied this with 4, assuming a 4h low tide period during the daylight period. This gives an annual estimate of the benthic primary production ranging from 0.86 to 12 g C m\(^{-2}\). These ranges of primary production are lower compared to other estimates of benthic primary production which varied between 62 and 276 g C m\(^{-2}\) yr\(^{-1}\) in the Wadden Sea (Colijn & de Jonge, 1984, Philippart & Epping, 2010). Compared to the MPB primary production estimated in this study, higher annual primary production rates that were observed in the Ems-Dollard estuary by Colijn & de Jonge (1984) were attributed to MPB communities on muddy sediment. The difference between the rates of MPB primary production measured in this study and those reported in the literature can be partly explained by MPB in muddy sediments fix more C because of their apparent higher activity and higher densities (Billerbeck et al., 2007). Alternatively, patchiness of the MPB biomass in the tidal flats or methodologies for primary production measurements could also explain differences in primary production. If we had measured in the mussel beds on the Balgzand tidal flat (where TfS20 was located), we might have measured much higher primary production rates because the MPB communities in these muddy sediments were dense (personal observations). However, the sites that we selected were, at least by eye, more representative for most of the surface area of the tidal flats.

The Marsdiep basin has extensive intertidal flats with a total surface area of 9451 ha (range of low water levels +65 to −77 cm), the annual average high and low tide area, whereas the total surface area of the Marsdiep basin is 67230 ha. Therefore, the benthic primary production ranges from 38 to 538 tons of C per year, whereas the phytoplankton production ranged from 954 to 31895 tons of C per year in the Marsdiep basin. Although the primary production of the MPB per unit area can exceed the phytoplankton primary production, the overall contribution of the MPB to the total aquatic primary production is also dependent on the morphology of the system.
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Tables

Table 1. Coordinates of the pelagic (S18, S19 and S20) and benthic stations (TfS18, TfS20 and TfS21) at different sampling periods (April: Nav7, May/June: Nav8 and September: Nav9 2011)

<table>
<thead>
<tr>
<th>Navicula sampling</th>
<th>Stations</th>
<th>Type</th>
<th>Coordinates (Lat N)</th>
<th>Coordinates (Long E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nav7</td>
<td>S18</td>
<td>Pelagic</td>
<td>53° 03.136'</td>
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Table 2. Primers used and target sites

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Table 3. PCR programs conditions of the nested PCR (10 steps in which annealing temperature decreased with -0.5 °C per step; two separate reactions, later combined)

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<th>PCR CONDITIONS</th>
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<th>Annealing time</th>
<th>Annealing ° C</th>
<th>Extension time</th>
<th>Extension ° C</th>
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Figures

Figure 1. Map of Marsdiep basin (pelagic stations S18, S19 and S20; benthic stations: TfS18, TfS20 and TfS21) (NL: the Netherlands).
Figure 2. Cluster analysis of DGGE based on OTU presence of 16 S RNA-Cyanobacteria community (A) and 18 S RNA-Eukarya community (B) 15 samples during three sampling seasons (April: Nav7, May/June: Nav8 and September: Nav9 2011) at three pelagic stations (S18, S19 and S20) and two benthic stations (TfS20 and TfS21).
Figure 3. Shannon’s diversity index (H) based on OTU presence: 16S rRNA (A) and 18S rRNA (B) during three sampling seasons (April: Nav7, May/June: Nav8 and September: Nav9 2011) at three pelagic stations (S18, S19 and S20) and two benthic stations (TfS20 and TfS21).
Figure 4. $^{13}$C-fixation into bulk into organic matter (mg C m$^{-2}$ h$^{-1}$) during three sampling seasons (April: Nav7, May/June: Nav8 and September: Nav9 2011) at three pelagic stations (S18, S19 and S20) and two benthic stations (TfS20 and TfS21). Data are represented as average with SD errors.