Final project report
Reporting template

Project acronym		BIO-Tide
Project title		The role of microbial biodiversity in the functioning of marine tidal flat sediments
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	Entity (Company/organization)	Ghent University
Project period (Start date – End date)		01 March 2017- 29 Feb 2020 (30 Nov 2020)
Project website, if applicable		https://sites.google.com/view/biotide

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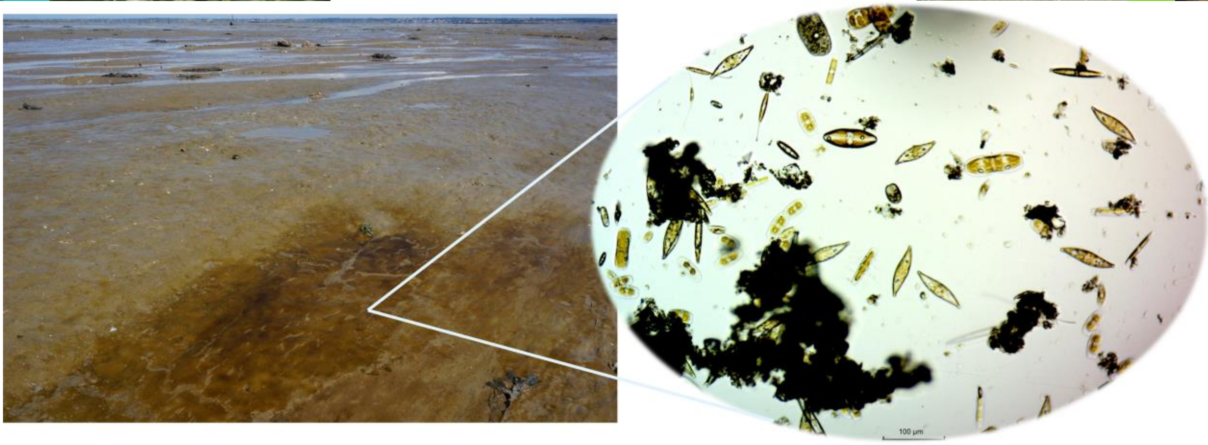
List of partners involved in the project (company/organization and principal investigator). Please use partner numbers to specify the tasks, work packages and inputs of each partner in sections 4.3, 5 and 6.2 to 6.4.	Partner 1: UG-PAE (Ghent University, Protistology & Aquatic Ecology, BE) Partner 2: LAUS (Ecole Polytechnique de Lausanne, CH) Partner 3: UG-MB (Ghent University, Marine Biology, BE) Partner 4: MNHN-BOREA (Muséum National d'Histoire Naturelle - UMR BOREA, FR) Partner 5: NANT (Université de Nantes, FR) Partner 6: ST-AN (St-Andrews University, UK)

1. Short description for publicity

At first sight, coastal tidal flats appear to be barren expanses of mud and sand. A closer look however reveals that they are teaming with life. Minute tracks and holes betray the presence of worms, crustaceans and shellfish, which in turn are eaten by fish, migratory birds and humans. In the BIO-Tide project, we show that this rich diversity of animal life is supported by highly productive biofilms which are dominated by microalgae. At low tide, these biofilms form intense brown patches at the mud surface. Using photosynthesis, the microalgae produce organic carbon which is rapidly taken up in the whole food web. We showed that the biodiversity of these biofilms has a strong impact on their functioning, such as carbon cycling, and that this effect is mediated by sophisticated interactions between different microbial life forms, from bacteria and microalgae to minute roundworms. Tidal environments are also ideal sites for aquaculture, as the biofilms also represent a highly nutritious food source for commercially important animals such as oysters and sea urchins. Studying tidal flats however is not easy, as they are often very inaccessible and sometimes even dangerous. We therefore developed tools which allow documenting the biodiversity and ecosystem functioning of whole tidal flats from the air ('remote sensing'), using drones, planes and even satellites. Taken together, our results show that the healthy functioning of these important coastal systems depends on the biodiversity of the microbial biofilms that inhabit the tidal flats.



Microalgal biofilms forming a dense, brown layer on the mudflat at La Coupelasse (F), where the first BIO-Tide campaign took place in June 2017. The inset shows the high biodiversity of benthic microalgae which dominate the biofilm.

Benthic microalgae drive mudflat ecosystem functioning. Their intense photosynthesis leads to the formation of oxygen bubbles by the biofilms.

2. Summary

Tidal flats are inhabited by dense microalgal biofilms, which are highly productive and fuel tidal flat and coastal food webs. While it has been shown that the microbial biodiversity in these biofilms is huge, to date very little is known about how it affects the functioning of tidal flat ecosystems. In the BIO-Tide project, we used a combination of state-of-the-art ecophysiological, biochemical, stable isotope probing, omics, remote sensing and modeling tools in field and lab settings to elucidate the biodiversity-ecosystem functioning (EF) relationship in tidal flats.

During field campaigns in France and The Netherlands, we studied short-term (tidal to diurnal) variability in biodiversity and EF in contrasting sediment types. While silt had higher biofilm biomass (dominated by benthic microalgae and bacteria) and more intense C cycling (higher production and respiration), the sandy site had higher microbial diversity and a more specialized food web. We showed that rapid micro-scale vertical migratory rhythms help to sustain high microalgal primary production. Surprisingly, food web modeling showed that while most benthic animals directly or indirectly derive a substantial part of their C from the microalgae, the microalgae-meiofauna pathway is stronger than the microalgae-macrofauna pathway. In the second field campaign, we combined meta-omics with a novel untargeted metabolomic approach to elucidate metabolic pathways and products (inluding extracellular polymeric substances) at high taxonomic resolution. Results so far reveal a marked diversity and temporal variability in hitherto underreported metabolites. These will be

linked to community gene expression data to evaluate how the biochemical diversity is related to species diversity.

We demonstrated that microalgal diversity has a significant impact on EF, and this is modulated by species-specific interactions with bacteria and meiobenthos. Different microalgae differ in their photosynthetic and mixotrophic capacities, suggesting that resource-related niche differentiation is important. Species-specific interactions between microalgae and bacteria suggest co-adaptation which enhances synergism. Meiofauna were shown to stimulate biofilm growth through grazing and bioturbation (bio-irrigation).

In collaboration with private stakeholders, we successfully applied BIO-Tide knowlegde in aquaculture, and developed novel synoptic remote sensing tools to model biofilm primary production over a wide range of spatial scales. We showed that biofilm microalgae can be a game changer for sea urchin and sea cucumber aquaculture. The above-mentioned new metabolomic approach allows rapid assessment of metabolic diversity including interesting molecules which may be of relevance for aquaculture. We initiated the development of a novel type of photobioreactor for growing benthic microalgae. Finally, the newly developed remote sensing tool will allow more accurately estimating tidal flat primary production and will therefore be highly valuable to coastal stakeholders and managers.

3. Objectives of the research

The overarching objective of the BIO-Tide project was to identify and quantify the relation between microbial biodiversity and C cycle related ecosystem functions (EFs) in contrasting tidal flat environments (sand vs silt) in the context of biotic interactions.

(1) To identify and quantify the relation between biodiversity, the C cycle and sediment stability in tidal flat sediments across multiple food web levels in contrasting sediment types (WP1-4).
(2) To determine and quantify the importance of bacterial and benthic microalgal (BMA) biodiversity for extracellular polymeric substance (EPS) composition and dynamics, and sediment stability (WP1,2). This objective has been broadened to also include effects of BMA-bacteria interactions on other C cycling related EFs (i.c. biomass production) and to include hitherto understudied metabolites.
(3) To determine the quantitive importance of BMA mixotrophy and the role of BMA diversity in this process (WP2). This objective was broadened to also incorporate the potential effect of bacteria on C assimilation capacities in diatoms.
(4) To determine and quantify the effect of meiobenthos diversity on C cycling and sediment stability via bioturbation and grazing (WP3).
(5) To assess and quantify the effect of BMA diversity on the productivity of a commercial suspension feeder (the oyster *Crassostrea gigas*)(WP4). In consultation with stakeholder Benth'ostrea it was decided to focus on sea urchins instead of oysters, although experiments with oysters were also performed. In addition, a pilot study on the use of benthic microalgae in sea cucumber cultivation was performed, and lipid production in a diverse selection of BMA was explored.
(6) To reconstruct and quantify C flows through the tidal flat ecosystem at high biodiversity resolution using linear inverse models (WP5).
(7) To explore to what degree we can use remote sensing (RS) techniques to scale up our field, experimental and modeling results to the level of whole tidal flat systems (WP6).
(8) To closely interact, throughout the project lifetime, with stakeholders in order to tailor specific research questions and tool development (models and RS) to their needs (WP7, all WPs).

Please note that throughout the report, manuscript numbers (MXX) are used for referencing to published papers resulting from the project. The manuscripts and their numbers are listed in the table in section 6.1.

4.1. General description of activities over the duration of the project

Objectives 1- 4 were addressed in two field campaigns and dedicated lab experiments. The field campaigns took place at tidal flat sites in France (La Coupelasse, Baie of Bourgneuf, June 2017) and The Netherlands (Biezelingse Ham, Westerschelde estuary, June-July 2018). All partners (except P2 in 2017 and 2018, and P6 in 2017) participated to both campaigns. Central to the **2017** campaign was a large-scale **stable isotope probing (SIP) pulse-chase experiment** carried out in two contrasting sediment types (mud vs sand). At t=0, sediments were sprayed with sodium ^{13}C-bicarbonate. Samples were then collected each hour for 4 (mud) to 3 hrs (sand)(pulse period) and then again after 24, 48 and 120 hrs (chase period). Samples were taken for abiotic (T°C, salinity, sediment grain size, water content, porosity and nutrient pools, P1,3) and biotic parameters (TOC, bulk carbohydrates, EPS, pigments, fatty acids, spectral reflectance, P1,3-5) and biodiversity assessments (pigments, 16S and 18S rDNA and rRNA amplicon sequencing, meio- and macrobenthos, P1,3,5, BIO-LITTORAL). SIP analyses were performed for DIC, TOC, meio- and macrobenthos, fatty acids, EPS and RNA (P1, 3-5). Primary production (PP) and community respiration were measured using PAM fluorometry (P5) and CO_2 flux measurements (P4). BMA vertical migration was measured using microscopy (lens-tissue), spectral reflectance and PAM (P1, 5). Sediment stability and erosion thresholds were measured using a cohesive strength meter (P1). As the RNA-SIP approach used in the 2017 campaign was not successful (see 4.3), we opted for a **detailed metatranscriptomic and metabolomic approach** in the **2018** campaign. One muddy site was sampled throughout two consecutive low tides (day, night). Metabolomics was performed using an untargeted metabolomic approach for BMA biofilms developed by P4 (M11). The same abiotic and biotic parameters and biodiversity assessments (as in the 2017 campaign) were made by the same partners. In addition, light and oxygen profiles were established (P5), and vertical profiles of oxygen, pigments, nutrients and organic C were established to model organic matter degradation and nutrient remineralisation during a diurnal tidal cycle (P1,3). EFs include PP (P4,5), community respiration (P4,5), organic matter degradation, nutrient remineralisation (P1,3), sediment stability (P6), vertical migration (P1,5) and enzymatic activity (P4). The 2017 field campaign data sets are almost complete, but measurements for the 2018 campaign are still in progress. It was originally planned to hold a project meeting in early spring 2020 to discuss, integrate and prepare data for joint publications on the field campaign data, but the covid-19 situation has severely impeded progress (see also 4.5).

In addition to the two main campaigns, monthly samplings were carried out at the French site (muddy and sandy station, March 2017-July 2019) to determine how seasonal changes in BMA biodiversity affect PP, pigment diversity and EPS diversity [objective 2, P1,4,5, in collaboration with academic stakeholders Geslin and Kühl (see 5.2)]. These data are currently being prepared for publication. This field work was complemented with experiments to assess the impact of sediment type and light on BMA PP and photo-regulation (P4,5, M13) Furthermore, in collaboration with the two above-mentioned scientific stakeholders a manuscript will soon be submitted on the interaction between benthic diatoms and kleptoplastidic foraminifera, and a paper on the impact of epipelic diatom biodiversity on their photo-regulation mechanisms is being prepared.

Biotic **interactions between BMA and bacteria**, and how these affect ecosystem functioning, were studied in dedicated lab experiments (objectives 2-3, P1,2,4,5). In a first set of experiments, the effect of bacterial isolates on the growth of individual diatom strains was studied in order to evaluate the nature and specificity of the diatom-bacteria interactions. In a second set of experiments, the effect of bacteria on diatom community composition and vice versa, and how this influences the BEF relation, was investigated. In a third experiment, an axenic diatom strain was exposed to a foreign and a familiar bacterial inoculum, after which the cocultures were allowed to develop over 8 consecutive (diatom) growth cycles. At the end of the experiment, differential recruitment of bacterial assemblages and their effect on diatom growth were assessed (metabarcoding, ecophysiological characterization). In addition, metagenomics and -transcriptomics were used to functionally characterize the diatom-bacteria interaction, and SIP was applied to study the C flux from diatom to bacteria. Finally, the impact of bacteria on C assimilation strategies (photo- vs heterotrophy) in different BMA species was addressed using nanoSIMS (P1,2,5). The main aim of the latter experiment was to study **mixotrophic** capacities of different BMA species by feeding them with ^{13}C labelled $NaHCO_3$ or EPS and using nanoSIMS to quantify species-specific uptake of these labels in natural communities (objective 3, P1,2,5). The results of these experiments have been published (M3, 5) or are being prepared for publication.

The **effect of meiobenthos diversity on C cycling and sediment stability** via bioturbation and grazing was quantified using field (cf. above) and lab experiments (objective 4, P1,3). The results of the 2017 SIP field experiment were used to quantify C uptake through BMA grazing by both meio- and macrobenthos at high taxonomic resolution (species to genus level). These data are included in the linear inverse model (objective 6) to allow quantification of the effect of nematodes on benthic C flows. Different simulations in which the contributions of one or more species are removed and substituted by surplus contributions of the remaining taxa (as a simulation of species loss without concomitant loss in abundance) will shed light on the possible effect of biodiversity on the contribution of nematodes to tidal flat C flows. Three experiments were set up to study the effect of meiobenthos grazing and bioturbation on C cycling (P1,3). In a first experiment, the effect of a natural nematode community on the productivity and EPS production of an artificial BMA biofilm were studied using a new design of tidal microscosm (M1). In a second experiment, in collaboration with a new academic stakeholder (Cnudde, UGent, B), we developed a novel approach using a combination of X-ray radiography and computed tomography scanning of intact sediment cores to characterize bioturbation activity of different nematode species (D'Hondt et al., in prep.). Finally, using this set-up we investigated the relationship between nematode abundance and (micro)bioturbation. Results of these experiments allow evaluating to what extent nematode functional diversity affects biofilm EFs.

Upon request of private stakeholder Benth'ostrea it was decided to test the **impact of diatom diversity on sea urchin production** instead of on oysters (**objective 5**). More specifically, the effect of diatom diversity on the recruitment of the larvae of the sea urchin *Paracentrotus lividus* was studied (P5, Benth'ostrea, M9). This work was followed up by further experiments in 2019 with the diatom species that gave the best results, and also including the effect of antibiotics, oyster shell particles and a natural BMA biofilm obtained. This follow-up work is still ongoing but has been temporarily halted by the COVID-19 pandemic. P4 was involved in a study to assess the importance of BMA as a food source of sea cucumbers. In collaboration with P1, P5 assessed the **effect of resuspended sediment on oyster feeding**. Clearance rates of two diatom species by the oysters were studied at different sediment concentrations. The first results were inconclusive, and follow-up activities have been suspended because of COVID-19. In addition, the **potential of exploiting benthic diatom diversity for the production of commercially interesting lipids** (PUFA, ARA and EPA) was investigated (P5, M10). Lipid production of selected promising species was studied in relation to light and nutrient stress. Realizing that existing photobioreactors are not ideally suited for growing benthic diatoms, a promising collaboration with a new private stakeholder (the biotech start-up Synoxis Algae) was initiated to design a modular photo-bioreactor that can be used to optimize growth of benthic diatoms.

Objective 6 was addressed by developing two highly resolved food web models using **linear inverse modelling**. The models were developed by P3 with benthic biomass data collected and fluxes measured by P1, 3-5 during the 2017 field campaign. The models incorporated organic matter, bacteria, several primary producers, microbenthos (i.e., ciliates), meiobenthos (nematodes on genus level, other meiofauna taxa on higher taxonomic resolution), and macrobenthos at species level (Stratmann et al., in prep.). In addition, the early diagenesis of organic matter during a tidal cycle at night and a tidal cycle during the day will be modelled using the OMEXDIA approach (developed by P3, with input data collected and measured by P1,3-5 during the 2018 field campaign). This will allow assessing how organic matter degradation and nutrient remineralization is affected by tides and the day-night cycle.

Remote sensing (RS) approaches in combination with modeling and experimentation were used to assess and map sediment type, BMA gross primary production (GPP) and BMA resuspension (**objective 7**, P5). The objective of mapping sediment type using multispectral imagery was not successful. Several existing models were tested on SPOT and Pléiades images to map the mud content of sediment. Mapping GPP using multispectral imagery (SPOT and Pléiades) and hyperspectral imagery (HySpex) was performed by coupling RS, modeling and experimentation (M12,14); the application of hyperspectral imagery is being further developed (PhD M. Zhang). Mechanisms underpinning BMA resuspension at ecosystem-scale were investigated using a coupled physical-biological model forced by realistic meteorological and hydrodynamical forcings to simulate chronic (without any concomitant sediment resuspension) and massive (driven by bed failure) BMA resuspension. Modeled estimates of resuspended BMA Chl a were compared with (simultaneously acquired) Chl a field data and RS-derived Chl a estimates. RS-derived Chl a was computed using a semi-analytical inversion algorithm specifically developed for coastal and inland waters specifically tuned to turbid intertidal waters (Gernez et al., 2017).

Stakeholder interaction (**objective 8**) is described in detail in section 5 of this report.

4.2. Table of deliverables

Deliverable and Milestone Name			Lead partner	Date of delivery		Comments
				Planned	Delivered	
Work Package	**Del/Mil**	**Full Name**				
WP1	D05	Data sets and manuscript(s) on field campaign measurements of microbial, meio- and macrobenthos taxonomic and functional diversity, C fluxes and sediment stability, and the relation between these variables in Baie of Bourgneuf tidal flats	P1	04/2018	Partly delivered on time, ongoing	Complete data sets have been produced. Joint publications are in progress.
	D09	Data sets and manuscript(s) on field campaign measurements of microbial, meio- and macrobenthos taxonomic and functional diversity, C fluxes and sediment stability, and the relation between these variables in the Biezelingse Ham tidal flat.	P1	04/2019	Partly delivered on time, ongoing	As RNA-SIP (2017 campaign) did not deliver satisfactory results, we opted for a metatranscriptomic approach in the 2018 campaign. Biezelingse Ham was selected as location instead of Paulina tidal flat. Omics analyses are ongoing. Joint papers are in progress.
	D10	Manuscript(s) of EPS composition and dynamics in sandy and silty tidal flats	P5	04/2019	Partly delivered on time, ongoing	These data will be included in joint manuscripts on the 2017 campaign. A new method to determine EPS and metabolite composition in marine sediments was been developed and published by P4 (M11). The protocol has been applied to the 2018 campaign data, and the results will be incorporated with the metatranscriptomic data in a joint publication (Gaubert-Boussarie & Bogorad et al., in prep.).
	D11	Stable isotope labelling data sets and manuscript(s) based on CSIA and RNA-SIP	P3, P1	04/2019	Partly delivered on time, ongoing	All stable isotope data have been delivered and are being included in the linear inverse modelling of carbon flows in the food web (Stratmann, in prep.).
	M04	1st field campaign debriefing, upload first field data in data management system	P1	07/2017	07/2018	
	M05	Submission of first manuscript (see deliverables: further submission continuously throughout project, all WPs)	P1	03/2018	03/2018	In 2018, the first publications were published (M1, Hubas et al. 2018, Passarelli et al. 2018, Moens & Beninger 2018, Van Colen 2018, the latter four are chapters in the book “Mudflat Ecology” (Springer, edited by P5) and involving P3-6.
	M07	2nd field campaign debriefing	P1	07/2018	07/2018	

WP2/WP3	*D06*	*NanoSIMS protocol for studying microbial BEF relations in intertidal sediment biofilms + manuscript*	*P2, P5*	*09/2018*	*Delayed, ongoing*	*The fine-tuning of a nanoSIMS protocol (P2,5, stakeholder Prof. E. Geslin, Univ. Angers) took longer than expected. This task was delayed for a number of reasons: (1) the postdoc responsible for this task left the project after three months, and had to be replaced. (2) the resin we tested to keep the sediment structure intact did not work. (3) We also underestimated the problem of diatom frustule thickness, leading to a considerable increase in nanoSIMS operating time. A first draft of a manuscript has been written and a final version should be completed in the next months. In addition, an experiment has been performed on the uptake of DOM by benthic nematodes using NanoSIMS (collaboration between P3 and University of Utrecht, NL); these data are currently being analysed and prepared for publication (Guilini et al. in prep.).*
	D08	*D.08 – Gallery of microscope, FISH and NanoSIMS images illustrating bacteria & BMA diversity*	*P2, P5*	*01/2019*	*Delayed, ongoing*	*SEMS and nanoSIMS images of various BMA species, sampled at the Paulina tidal flat have been taken, have been produced. SEM images also contain meiobenthos (nematodes). Unfortunately, the nanoSIMS protocol did not permit to image bacteria.*
	D12	*Data and manuscript(s) on the importance of diatom diversity in photo- and mixotrophic C flux in tidal sediments*	*P1*	*04/2019*	*Partly delivered on time.*	*Experiments were conducted (P1,2,5) on the uptake of ^{13}C labelled inorganic and organic (EPS) carbon by various BMA species, and the impact of light and bacteria on C assimilation pathways. A first draft of a manuscript has been written and a paper should be finalized in the next months (Hubas et al., in prep.).*
	D13	*Data and manuscript on the relation between diatom diversity and EPS composition and functionality*	*P1*	*04/2019*	*Partly delivered on time, ongoing*	*Detailed data on EPS composition and functionality were obtained for the 2018 campaign (P4). These data will be compared with BMA molecular-taxonomic diversity (P1). These data will either be included in a more general joint paper on the 2018 campaign, or will be published in a separate paper.*
	D14	*Nematode grazing rates at low and high diatom diversity: data and manuscript*	*P3*	*04/2019*	*04/2020*	*The originally conceived experiment has not been implemented as such. Data on in situ grazing rates from the first field experiment have been completed and are included in the linear inverse modelling of carbon flows in the food web (P3, all partners, joint paper in progress (Stratmann et al. In prep.).*

	D15	*Nematode bioturbation rates: data and manuscript*	*P3, P1*	*07/2019*	*Data completed on time, manuscripts delayed but in progress*	*A manuscript (M1) on the bioturbation of nematodes belonging to different 'functional groups' is in progress and will be submitted in January 2021. A second manuscript looking into the influence of nematode abundance on bioturbation rate is in preparation (D'hondt et al. In prep.).*
	D20	*Data and manuscript(s) on the effect of bacterial and diatom diversity on bacterial C uptake in tidal sediments*	*P1*	*01/2020*	*Data completed, analysis and manuscript in progress*	*A joint experiment (P1,4,5) was set up including metatranscriptomics. The latter data are still being analyzed and will be prepared for a manuscript in the coming months (Bogorad, in prep.).*
	D23	*Data and manuscript on the effect of nematode herbivory and bioturbation on the microbial BEF relation*	*P3,*	*03/2020*	*Data completed on time, manuscript in preparation*	*A manuscript on the effects of a nematode community on microphytobenthos biofilm biomass accumulation and species composition, and on EPS production and bacterial community composition, has been published (M1). Two manuscripts on nematode bioturbation effects are planned under D15, whilst nematode grazing rates are planned to be part of a joint paper on the 2017 field experiment (see D05, D14, D21 & D22).*
WP4	*D07*	*Data and manuscript on effects of effect of diatom identity and diversity on oyster production*	*P5*	*10/2019*	*10/2019*	*In consultation with private stakeholder Benth'Ostrea focus was shifted to sea urchins. A manuscript has been published (M9). In consultation with the Holofarm consortium including AQUA-B stakeholder, attention was also shed on the sea cucumber Holothuria forskali (David et al 2020).*
	D16	*Data and manuscript on interference of oyster feeding on diatoms by suspended particles*	*P5*	*07/2020*	*07/2020*	*An experiment was set up and analysed in collaboration with P1. However, the results were not usable.*
	D24	*List of diatoms with high potential for oyster feeding. This task (see also objective 5, section 3) was slightly modified in a more general evaluation of the potential nutritional quality (lipid production) of benthic diatoms.*	*P5*	*03/2020*	*03/2020*	*These analyses were published (M10)*
WP5	*D21*	*Inverse models (+ manuscript) describing sedimentary C cycle*	*P3,P1*	*01/2020*	*Ongoing*	*The results from D21 and D22 will be published together in one article (Stratmann et al. in prep.). Model development is slightly delayed, as all SIP analyses have only just been finalized.*
	D22	*Dynamic tracer uptake model (+ manuscript)*	*P3,P1*	*01/2020*	*Ongoing*	*See comment for D21.*
WP6	*D17*	*Maps of sediment type and water content based on remote sensing data*	*P5*	*10/2020*	*10/2020*	*14 maps were produced using Daggers et al. (2018) algorithms (unpublished)*
	D18	*Maps of BMA biomass and production based on remote sensing data: maps + manuscript*	*P5*	*10/2020*	*10/2020*	*These data were published in two papers (M12, 14).*
	D19	*Algorithms (+ manuscript) for deducing BMA primary production from spectral reflectance data*	*P5*	*10/2020*	*Partly delivered on time, ongoing*	*These data were partly published (M2), parts of the analyses on the use of hyperspectral data are still ongoing (PhD M. Zhang).*

	D25	Data (and manuscript) on the spatial link between BMA biomass and water column biomass	P5	03/2020	03/2020	These data were published (M4).
WP7	D01	Project website & social media accounts (Facebook, twitter, etc.)	P4	05/2017	05/2017	A dedicated website is available (https://sites.google.com/view/biotide) and several events of the BIO-TIDE project are communicated to the general audience via laboratory twitter accounts (@UMR-BOREA and @rsbe_MMS, e.g. https://twitter.com/rsbe_MMS/status/966317759557722112 or https://twitter.com/UMR_BOREA/status/1013412148574408705). In addition, a project page has been published on the social network ResearchGate (https://www.researchgate.net/project/BIO-TIDE).
	D02	Outline of strategy and concrete plan for outreach activities	P1	05/2017	05/2017	See section 5 for details
	D03	Stakeholder newsletters (annual from month 12)	P1	03/2018	abandoned	This deliverable was abandoned in favour of more concrete interactions with selected, involved stakeholders.
	D04	Printing promotional materials (leaflets, posters for outreach events)	P1	03/2018	Delivered and ongoing	BIO-Tide posters and presentations have been presented at several scientific meetings (see section 6).
	M01	Kick-off meeting	P1	03/2017	03/2017	
	M02	Launch project website and social media accounts	P2	05/2017	05/2017	Cf D01
	M03	Launch of project data management system	P1	05/2017	04/2018	The data management system was introduced by collaboration-stakeholder VLIZ at the 2nd progress meeting (April 2018).
	M06	Publication first stakeholder newsletters (annual from month 12)	P1	03/2018	abandoned	Cf. D03
	M08	First annual meeting, including stakeholder session	P1	06/2018	04/2018	
	M09	Second annual meeting, including stakeholder and outreach session	P1	03/2019	04/2019	No outreach session, abandoned in favour of more concrete interactions with selected, involved stakeholders
	M10	Final meeting with outreach event ('open day')	P1	03/2020	Postponed	Postponed to Jan 2021 because of Covid-19 pandemic. No outreach event will be planned, but several outreach events to the public at large have been done during the project (cf. section 5).

All objectives have been achieved (see below, 4.1, 4.2). Some tasks have been slightly modified [e.g. we used a metatranscriptomic not an RNA-SIP approach in the 2018 field campaign (WP1); experiments were performed with sea urchins not oysters in WP4]. Fifteen papers have already been published, 1 is submitted and 19 papers are being prepared. The fact that no in person meetings could proceed because of the COVID-19 pandemic (e.g. planned meeting in March 2020 between P1,3 and 5, final project meeting, etc.) has caused a significant delay in the preparation of joint publications.

Below, the principal outcomes of the project are discussed per WP. Because of space restrictions, no figures or tables could be added.

WP1 – Integrated field campaigns (all partners except P2)

The two field campaigns represented a major collaborative effort and generated a wealth of detailed data sets on microbial biodiversity and ecosystem functioning (EF). Data analysis (especially the SIP data) for the 2017 campaign took longer than foreseen, and joint papers are being prepared (e.g. Stratmann et al., in prep.). Most data for the 2018 campaign are ready, but analysis and data preparation are still ongoing.

In the 2017 campaign, major differences were observed in biodiversity and EF of the silty and sandy site. Spectral reflectance and pigment analyses showed that BMA biomass (~ NDVI, chl a) was higher at the silty site. rDNA and rRNA amplicon sequencing revealed higher pro- and eukaryotic mictobial diversity in the sandy site, with different diatom communities dominating BMA activity at both sites (confirmed by the reflectance and pigment data). Prokaryotic activity at the silty site was, surprisingly, dominated by the cyanobacterium *Planktothricoides sp.*, while at the sandy site alpha- and gammaproteobacteria were codominant. Silty sediments were more productive than sandy ones (based on CO_2 fluxes). Variation in NDVI values during low tide was higher at the muddy site, revealing the importance of BMA vertical migration. Pronounced changes in prokaryotic activity took place during tidal emersion, with an increasing importance of heterotrophic activity (possibly related to BMA EPS production).

The ^{13}C pulse-chase experiment was, apart from the RNA-SIP, successful. The inorganic ^{13}C was rapidly taken up at both the sandy and the muddy site and assimilated by the different benthic organisms inhabiting the sediment. The sandy site incorporated less ^{13}C than the silty one, but due to the lower C standing stock, proportional enrichment was much higher. Based on ^{13}C incorporation of group specific fatty acids, C fixation at both sites was largely due to the diatoms and cyanobacteria, confirming the rRNA data. C fixation by chemoautotrophic bacteria was also detected at both sites. The C fixed by phototrophs was rapidly transferred to the other trophic levels and to the EPS. Within one hour after labelling, the colloidal and bound factions of the EPS in the sandy site were respectively composed of 12% and 2% ^{13}C, at the muddy site 8% and 3.5% ^{13}C. Nematode species diversity in both sediments was low, with no more than five species together accounting for > 75% of abundance. Nematode abundance was > one order of magnitude higher in the silty sediment. Specific ^{13}C uptake was highest in deposit-feeding and epistratum-feeding nematodes, which both use BMA. However, there was also clear ^{13}C enrichment in ciliate feeders and predators of other nematodes, suggesting a rapid trophic transfer of fresh BMA production. In addition, the nematode *Terschellingia longicaudata*, which derives most of its C from chemoautotrophy, also took up ^{13}C, confirming that chemoautotrophic production in the silty sediment was significant. Towards the end of the low tide, specific uptake in nematodes was similar in the silty and sandy sediment. By the next low tide however, specific uptake further increased in silty sediment nematodes, whereas it decreased in the sandy ones. This most probably reflects erosion/resuspension of labelled BMA and loss of unincorporated label during high tide in the sandy site (Middelburg et al., 2000). Strikingly, specific uptake of deposit-feeding and epistrate-feeding nematodes was up to one order of magnitude higher than that of macrobenthos.

Unfortunately, the RNA-SIP experiment did not yield usable results, as labeling [despite using high amounts of $NaH^{13}CO_3$ (10g per m^2)] resulted in insufficient enrichment and no clear heavy C signal could be obtained for most time points with the density gradient ultracentrifugations. As a result, we could not infer C flows at very high taxonomic resolution. Only a single potential ^{13}C photosynthetic incorporator, the diatom *Synedra* spp. (silty site only) could be detected based on the RNA-SIP results. The low ^{13}C incorporation of the RNA likely results from a diffuse C flow within both sediment types: C originating from remineralisation, internally stored C, cross-feeding and mixotropy dilute the signature of the ^{13}C pulse. For this reason, we decided to abandon the very labour-intensive and costly SIP approach in the 2018 campaign, and opted for a detailed omics approach instead.

For the 2018 campaign, we used a metatranscriptomic and metabolomic approach to study, at high taxonomic resolution, transcriptional activity of microbial pro- and eukaryotes in relation to environmental and functional changes in a mudflat BMA community throughout two consecutive low tides (day vs night). Amplicon sequencing revealed that prokaryotic activity (rRNA), both during day and night, was mainly dominated by alphaproteobacteria and cyanobacteria, while day and night eukaryotic activity was almost completely dominated by diatoms. Reflectance data showed that BMA biomass was high during the first 3hrs of low tide (NDVI up to 0.5), and was largely dominated by diatoms ($I_{Diatom} \approx 2.3 \times I_{Euglenid}$), after which it decreased (NDVI ~ 0.2) with a lower dominance of diatoms ($I_{Diatom} \approx 1.2 \times I_{Euglenid}$). Preliminary metatranscriptome analyses of a selection of samples revealed that day time emersion, photosystem II related genes dominated gene expression, while at night expression of C fixation related genes (esp. Rubisco) was significantly higher. The full metatranscriptome data set is currently being analyzed and will be integrated with the diversity, metabolomic and functioning data in a joint manuscript (Gaubert-Boussarie & Bogorad et al., in prep.). The untargeted metabolomics approach developed by P4 (M11) revealed a surprisingly high diversity of metabolites (including phytyl esters, lactones, specific alkanes and fatty acids) whose dynamics were significantly affected by light exposure as well as day night and tidal cycles. These molecules are known markers of stress, chloroplast senescence and/or cell-cell communication and may reflect the consumption of energy reserves, and their synthesis influences biofilm EF. These data will be compared with the amplicon and metatranscriptome sequencing data in order to assess how metabolite and taxonomic diversity and functioning are related, and will an unprecedented insight into BEF relations in BMA biofilms. Natural stable isotope data of EPS were analysed and a short communication about the origin of bound and colloidal EPS is being prepared (Hubas et al., in prep).

The results of the seasonal campaigns (La Coupelasse, P1,4,5) show how seasonal changes in BMA biodiversity affect PP, pigment and EPS diversity. Three manuscripts are currently being prepared. In a first paper, changes in epipsammic and epipelic diatom communities are compared over the course of two years, focusing on the role of biodiversity and the impact on PP patterns (Prins et al., in prep.). In a second paper, the effect of daily vertical migrations of the BMA biofilms on PP estimates, and how this changes with season (and associated shifts in BMA diversity) will be discussed (Prins et al. , in prep.). In a third manuscript, spatial and temporal variability in benthic kleptoplastidic foraminifera and its relationship with BMA diversity is analyzed (Jesus et al., in prep.). The seasonal field work also led to a follow-up experiment in which the impact of sediment type and light climate on BMA PP and photo-regulation was investigated (M13). Furthermore, a manuscript is close to submission on the relationship between benthic diatoms and kleptoplastidic foraminifera (Jesus et al., in prep.) and another on the impact of epipelic diatom biodiversity on their photo-regulation mechanisms (Prins et al. in prep).

WP2 – Effect of prokaryotic and diatom diversity on photo-, mixo- and heterotrophic C flow and sediment stability in experimental biofilms (P1, 2, 4, 5)

Different experiments were set up to address the effect of prokaryotic and BMA diversity in C cycle related EFs in tidal flat phototrophic biofilms, with focus on how interactions between prokaryotes and BMA affect BEF relations (P1,4). Our results clearly demonstrate that BMA (i.c. diatom) diversity and the interaction with bacteria has a significant impact on EF. The effect of different bacteria species on diatom functioning (i.c. biomass production) is highly bacteria and diatom species-specific, and in monospecific diatom cultures (*Cylindrotheca* and *Navicula* spp.), the effects on diatom growth were mostly neutral to antagonistic (M3,5). Antagonistic effects however were most pronouced with 'foreign' bacteria (i.e. bacteria which do not naturally co-occur with the diatoms, as opposed to 'familiar' bacteria), suggesting that co-adaptative processes result in more neutral and synergistic interactions (M5). In cocultures of axenic diatoms and bacterial inocula, different diatom species recruited different bacterial assemblages from mixed inocula (M3). In mixed diatom communities, a positive effect of diatom diversity on their biomass production was observed, mainly due to complementarity effects, and this effect is enhanced in the presence of bacteria. It is hypothesized that the latter may be due to bacteria differentially affecting BMA fitness, resulting in shifts in community structure and competitive balances, which enhances coexistence and biomass production (M3). However, higher BMA diversity is not related to higher bacteria diversity, suggesting that bacterial identity rather than diversity per se is important. Interestingly, while in several coculture experiments, antagonistic effects on diatom growth were initially strong (Stock, W., PhD thesis, Stock & Bogorad et al., in prep.), these effects appeared to rapidly attenuate or even convert to synergism after repeated coculture growth cycles, again suggesting that either through preferential recruitment of more synergistic strains or through co-adaptation, diatom-bacteria interactions tend to become positive (Stock, W., PhD thesis, Stock & Bogorad et al., in prep.). Our experiments also showed that diatoms are capable of recruiting, from a completely foreign inoculum, a bacterial assemblage that has a positive effect on diatom growth, suggesting that functional redundancy, at least with respect to diatom-bacteria interactions, is present. Metatranscriptomic, metabolomic and SIP data from diatom-bacteria cocultures inoculated with foreign vs familiar bacterial microbiota are still being analyzed and will be prepared for publication shortly (Stock & Bogorad et al., in prep.).

NanoSIMS was used to assess the use of different C assimilation strategies (photosynthesis vs mixo-/heterotrophy) in a selection of benthic diatoms in relation to light (light vs dark) and the presence of bacteria (axenic vs non-axenic)(P1,2,5). This experiment revealed that the tested diatoms differed in inorganic C uptake capacity in the light, and that while they all showed mixotrophic uptake of ^{13}C labelled diatom-derived EPS in light but especially darkness, they differed in this capacity. C assimilation was also differentially affected by the presence of bacteria. Interestingly, at least one diatom species (*N. phyllepta*) also showed inorganic C uptake in the dark, which may represent a strategy to alleviate DIC limitation of photosynthesis in dense BMA biofilms. To the best of our knowledge, this was the first time that nanoSIMS in applied to study C assimilation in a benthic diatom community. More detailed experimental analyses on EPS complexity and how this relates to EF (sediment stability, C flux, task 2.3), a.o. through specialized diatom-EPS-bacteria interactions, could not be addressed in the BIO-Tide project. However, they will be further studied in a follow-up project of P1 in collaboration with Prof. G. Underwood (Univ. Essex, UK)(see section 5.3).

WP3 – The impact and interaction of diatom and meiobenthos biodiversity on C cycling and sediment stability through herbivory and bioturbation (P1,3)

This part deals with dedicated experiments that were carried out to unravel the impact of the interaction between meiobenthos and BMA on EF in tidal flats. In addition, the 2017 SIP pulse-chase field experiment yielded data on C uptake through BMA grazing in meiobenthos at a very high level of taxonomic resolution (see WP1,5).

In order to perform experiments in which meiofauna, BMA and their interactions in incubations with controlled species composition/diversity under tidal conditions, we first designed a novel, modular type of benthic microcosm. Using these microcosms, we demonstrated that a natural nematode community stimulated diatom biomass accumulation and caused a shift in diatom community structure in a 4-species BMA biofilm (M1). Our observations underscore that nematodes are important for the structure and production of tidal biofilms and as such indirectly affect important biofilm-mediated EFs and services, such as sediment stability and biogeochemistry. We also demonstrated that nematodes affected the species composition of benthic prokaryotes. M1 is only the second study to demonstrate that nematodes can stimulate biomass and EPS production in BMA biofilms and the first to show concomitant shifts in diatom species composition. The stimulation of diatom biomass production could be related to grazing- or bioturbation-induced nutrient regeneration, or to shifts in interspecific interactions between diatoms (e.g. competition) as a consequence of selective grazing. Alternatively, nematode activity may shift the investment of BMA in cell biomass vs bound extracellular polymeric substances (EPS).

The bioturbation potential (BP) of tidal flat nematodes was investigated using a state-of-the-art combination of X-ray radiography to quantify nematode movement in intact sediments, and high-resolution X-ray computed microtomography (µCT) scanning of intact sediments to determine the mode and extent of sediment reworking by nematodes (D'Hondt et al., in prep.). Nematode motility strongly correlated with body length and also biomass, but not to other functional traits. It was largely confined to the upper mms of the sediment, which immediately constrains any bioturbation effects to a thin upper layer (this could be affected though to experimental manipulations and the low abundances of nematodes used in our experiments). We demonstrated considerable bioturbation by (only) the more motile nematode species. We found no evidence of vertical displacement of particles, nor did we observe any significant increase in sediment porosity, suggesting that biodiffusion is the principal mode of bioturbation by nematodes. This result is key to understanding how nematodes affect sediment biogeochemistry, which in turn impacts benthic BMA and prokaryotes. Whilst these results underline pronounced species-specific differences in BP between nematode species, we are currently analyzing the results of an experiment in which higher nematode abundances and a diverse, natural community were used to assess to what extent the BP of a natural nematode community can be predicted based on the BP's of the component species and/or on the abundance of nematodes (D'Hondt et al., in prep.).

WP4 - Effect of diatom diversity on sea urchin production; interaction with sediment resuspension (P1,5)

In consultation with private stakeholder Benth'Ostrea, experiments were carried out on sea urchins instead of oysters (P5, M9). We showed that sea urchin recruitment (*Paracentrotus lividus*) can be successfully promoted using diatom-based biofilms. Single diatom biofilms (*Nitzschia laevis*) are effective in inducing sea urchin metamorphosis, and form a practical and safe alternative to the use of natural biofilms for aquaculture, as it overcomes potential contamination.

The effect of sediment resuspension on oyster feeding rates was investigated by measuring oyster clearance rates with mixtures of two benthic diatom species (*N. laevis* and *Biremis lucens*) at a range of suspended matter (silt) concentrations (P1,5). Unfortunately, the results were not usable because the diatoms flocculated with the silts creating a background noise that did not allow measuring the clearance rate. Further experiments with added EDTA were planned but had to be abandoned because of the pandemic.

Experiments to explore the potential of diatom diversity for commercial oyster larvae rearing were not carried out. Instead, the production of commercially interest lipids (PUFA, ARA and EPA) in relation to light and nutrient stress was evaluated for a selection of benthic diatom strains (P5). After initial screening, three strains (*Entomoneis paludosa*, *Nitzschia alexandrina* and *Staurosira* sp.) were selected for a detailed analysis of their lipid production and growth capacities (M10). Using PAM fluorometry and Fourier-transform infrared (FTIR) spectroscopy, we showed that under high light and N limitation, the photosynthetic machinery is negatively impacted, leading to reduced growth and lipids and/or carbohydrates accumulation. However, increasing lipid content under stressful conditions does not increase their overall production, which is highest under favorable culture conditions.

WP5 – Inverse models of the sedimentary C cycle (P3, all partners)

Linear inverse modelling was used to develop highly resolved food web models for the sandy and silty site at La Coupelasse (2017 campaign (see objective 1, WP1, Stratmann et al., in prep.). Our models have a very high diversity resolution as taxa, and in particular meiobenthos, are usually lumped together in feeding types and not modelled as individual genera. Using taxon-specific physiological parameters (assimilation efficiency, biomass-specific respiration, production-biomass ratio) for macrofauna, our model was better constrained than previous food web models and we the first to dynamically model the incorporation of $NaH^{13}CO_3$ into BMA over time. Total bacteria, BMA, ciliate, meio- and macrofauna biomass was 2.7 times higher at the silty site (2,139±397 mmol C m^{-2}) than at the sandy site (801±338 mmol C m^{-2}). Most of the benthic biomass at the silty site consisted of sedimentary BMA (86.7%) and bacteria (9.12%), whereas ciliates, meio- and macrofauna contributed only 0.66 2.68 and 0.85% respectively. At the sandy site, bacteria contributed 45.8% and sedimentary microphytobenthos 49.9%. Preliminary modelling results showed that the total PP at the silty site (64.3 mmol C m^{-2} d^{-1}) was more than 2x higher than at the sandy site (29.6 mmol C m^{-2} d^{-1}) and consisted mainly of phytoplankton PP (49.9%) and sedimentary BMA PP (43.4%). In comparison, at the sandy site, phytoplankton, suspended BMA and sedimentary BMA contributed more evenly to total PP (38.8 28.9 and 32.3%). The ecosystem at the silty site was estimated to respire 63.8 mmol C m^{-2} d^{-1} with an 58.9% contribution by bivalves, 20.3% by bacteria, and 13.2% by meiofauna (except nematodes). The sandy site had an estimated total respiration of 29.5 mmol C m^{-2} d^{-1}, divided into non-nematode meiofauna (48.5%), bivalves (26%) and bacteria (15.9%). Preliminary modelling results show that at both sites BMA was an important food source for nematodes and other meiofauna. Additionally, parts of BMA at the silty site was deposited and contributed to the detritus pool, and was taken up by ciliates and bivalves.

First calculations show that the sum of all C flows in the food web (i.e., total system throughput) which can be interpreted as the 'ecological size' of the system, was 4.2x larger at the silty than at the sandy site. The number of food web compartments however was higher at the sandy site, with the individual taxa being more specialized resulting in a lower number of links and link density. Furthermore, at the silty site, where BMA biomass was > 4x higher than at the sandy site, Finn's cycling index was one order of magnitude higher compared implying that more C was recycled. Our modelling results are consistent with results from Marennes-Oléron Bay (France) mudflats where BMA was mainly assimilated by meio- instead of macrofauna, underscoring the importance of the BMA-meiofauna pathway. However, in comparison with food web models developed for the Channel coast (France), the food web compartments for the sandy and silty sites were connected via more food web links (180-361 vs 82-156) and the total amount of C flowing through the system was 2-10x higher.

For the 2018 campaign, dynamic biogeochemical, not food web, models will be developed (Stratmann et al., in prep.).

WP6 – Scaling up to the whole tidal flat system level using remote sensing (P5).

Remote sensing (RS) approaches in combination with modeling and experimentation were used to assess and map sediment type, BMA GPP and BMA resuspension. Several existing models were tested on multispectral SPOT and Pléiades images to map the mud content of sediment. However, these efforts were not successful. Inferred values ranged from -35 to 120%. Moreover, similar mud values (± 30%) were inferred for the sandy and muddy site at La Coupelasse (2017), which did not agree with *in situ* measured values.

BMA GPP was estimated from both multispectral and hyperspectral reflectance imagery. BMA GPP was estimated with a newly developed algorithm by coupling: (i) NDVI maps derived from high spatial resolution multispectral satellite images (SPOT6 or Pléiades); (ii) emersion time, photosynthetically active radiation (PAR), and surface temperature simulated from the physical model MARS-3D; (iii) photophysiological parameters retrieved from production–irradiance (P–E) curves, obtained under controlled conditions of PAR and temperature using benthic chambers. The novelty of this approach is the direct calibration of productivity using NDVI to be consistent with RS measurements of BMA biomass, leading to spatially upscaled GPP maps acquired in different seasons (M2). In addition, we developed algorithms to infer BMA GPP from hyperspectral *in situ* reflectance data. This was achieved by coupling hyperspectral radiometry (reflectance spectra, ρ and second derivative spectra, δδ) and PAM-fluorometry (non-sequential light curves). Five diatom species representative of the main BMA growth forms (epipelic, epipsammic and tychoplanktonic) were submitted to increasing light intensities (0-1950 µmol photons m^{-2} s^{-1}). Different fluorescence features were observed for the three growth forms which could be linked to their xanthophyll cycle. The optical cross section (a*) was retrieved using a radiative transfer model and several radiometric indices were tested for their capacity to predict light use efficiency (LUE) and photosynthetic electron transport rate (ETR) measured by PAM fluorometry. Only one radiometric index was not species- or growth-form specific, i.e. δδ496/508. This index was named MPBLUE and can be used to predict LUE and ETR (M2). The applicability of this index was tested to map ETR using an hyperspectral RS image acquired on the Bourgneuf bay mudflat (Méléder et al., in prep.). Reflectance, ρ and/or second derivative, δδ will be linked with BMA GPP (Zhang et al., in prep.).

Finally, RS imagery was used to study BMA resuspension at high tide. Both *in situ* and satellite observations compared well with the simulated resuspended Chl a obtained with a physical-biological coupled model (mud temperature, BMA biomass and hydrodynamics) forced by realistic meteorological and hydrodynamical forcings to simulate chronic (without any concomitant sediment resuspension) and massive (driven by bed failure) resuspension over a year (M4). The simulated maximum peaks of resuspended BMA (5–45 mg Chl a m^{-3}) generally compared well with satellite estimates (1.4–35.4 mg Chl a m^{-3}). The agreement between the satellite and *in situ* observation allowed us to better appraise the temporal dynamics of BMA resuspension using the model simulations. Over a year, 43% of the simulated BMA PP was resuspended, with BMA resuspension (60.8 g C m^{-2} yr^{-1}) exceeding the loss by grazing (41.1 g C m^{-2} yr^{-1}). At the seasonal scale, the export of BMA biomass was highest in spring (> 3x summer). Over a tidal cycle, BMA resuspension is the highest during flood due to high current velocity and low water height. All together, the results suggest that chronic and massive BMA resuspension events are important in the synoptic to seasonal BMA dynamics in temperate intertidal mudflats. Accounting for such processes in the C budget assessment in the land-ocean interface could bring new insights to our understanding of the role played by BMA in the coastal C cycle.

4.4. List of project meetings

Date	Place	Participating partners	Meeting title and object
6-7 march 2017	Nantes (Fr)	P1-P5	Kick-off meeting BIO-TIDE project, preparation first field campaign
4-5 April 2017	Brussels (B)	P1 & P5, joined by collaborator-stakeholder BIO-LITTORAL	BiodivERsA joint kick-off meeting, BiodivERsa Research and Innovation Workshop
20-22 June 2017	Nantes (Fr)	P1-P5, joined by collaborator-stakeholder BIO-LITTORAL and new stakeholder Univ. Angers (E. Geslin)	1st progress meeting, preparation first field campaign
9-10 April 2018	Ghent (B)	P1-P5, joined by collaborator-stakeholder VLIZ and Univ. Angers (E. Geslin).	2nd progress meeting, preparation second field campaign
4-5 April 2019	Ghent (B)	P1, P3-5	3rd progress meeting
13-14 Nov 2019	Brussels (B)	P1	Final conference of the research projects funded through the BiodivERsa 2015-2016 call and the Strategic Workshop on a possible Biodiversity Partnership and the co-organized by BiodivERsA and the European Commission (DG R&I, DG ENV)
Jan 2021	online	All partners	Project final meeting

In addition to these ‘official’ progress meetings, several *ad hoc* meetings between selected partners were held at various occasions, either dedicated meetings or meetings that piggybacked off other meetings. These included dedicated meetings between project partners (e.g. P1, 4-5 at HDR defence C. Hubas in Concarneau, F, 12/2018, P1, 4-5 at HDR defences B. Jesus and V. Méléder in Nantes, F, 10/2019, P1, 5 at 66Th Annual Meeting British Phycological Society & 3rd Microphytobenthos Meeting, Southend, UK, 01/2018, P1, 5 at 7th European Phycological Congress, Zagreb, HR, 09/2019, etc.), and between project partners and academic and private stakeholders (P1, 2 and academic stakeholder N. Geslin at PhD defence C. Lekieffre in Lausanne, CH, 09/2017, P5 and stakeholder N. Geslin at various occasions to discuss work on interactions between benthic diatoms and foraminifera, P5 and private stakeholder Benth’ostrea to organize work on sea urchins, P5 and private stakeholder Synoxis Algae to discuss the development of a biofilm-based photo-biorreactor, etc.).

4.5 Follow up activities and plans for further exploitation of the results

Several activities will be take place to ensure maximal application of the projects results:

(1) While numerous **papers** have already been published (see publication list), many others are still in progress. This is partly due to the fact that some data types take longer to be produced and processed than anticipated (especially omics and SIP data). Because these data are essential for the more integrated overview papers, most multi-partner, joint papers are still in preparation. Progress in this respect has also been significantly hampered by the COVID-19 pandemic, which led to the cancellation of planned in person meetings between the partners.
(2) BIO-Tide has enabled to consolidate existing interactions with both academic and private **stakeholders**, but has also paved the way for promising collaborations with new stakeholders (see 5). As outlined in more detail below, these concrete collaborations will be further developed in the future (e.g. use of BMA diversity in sea urchin and oyster aquaculture, development of photobioreactor for BMA cultivation).

(3) As outlined in the project proposal, tida flat ecosystems are hugely important in terms of numerous essential ecosystem services (ES) to coastal communities and beyond, and are therefore highly **policy-relevant**. The BIO-Tide project offers new insights and detailed knowledge on the microbial biodiversity status of tidal flat ecosystems in NW Europe, and how biodiversity affects their functioning and ES provision. This knowledge will guide **Integrated Coastal Zone Management (ICZM) strategies**, and as such are of great relevance to EU, national and regional policy. Our results convincingly show that the primary production of species-rich BMA biofilms forms the basis of the tidal flat food webs and controls fluxes of C to higher trophic levels, including commercially important species. This enhanced understanding is essential for the responsible integration of aquaculture activities in these areas. Moreover, the development of **food web models** and **new remote-sensing based tools** to map BMA primary production but also resuspension of BMA by tides, represents a major breakthrough for the (spatial) planning and management of multi-stakeholder activities in these ecosystems. Several BIO-Tide partners serve in team members in **advisory boards** for ICZM (see 5.2 and 5.3), ensuring effective transfer of BIO-Tide knowlegde and insights to policy makers. Following up on the final conference on projects of the BiodivERsa 2015-2016 call, we developed a **policy brief** ('Diverse and intact food webs are essential for maintaining tidal flat ecosystem services') which was allocated to maturity group 2. Unfortunately, further development of this brief got postponed due to the COVID-19 crisis. The BiodivERsa secretariat was contacted to see how we can best proceed with the further development of this brief.

(4) To date, BMA diversity remains largely underexploited in the context of microalgal cultivation for the production of interesting biomolecules for food and (aquaculture) feed. Our results show that benthic diatoms (which dominate BMA communities in temperate zones) represent a promising new source for these purposes (e.g. production of lipids, induction of sea urchin recruitment, etc.). Several partners (P1,4,5) are involved in new projects and project applications aimed at **exploring the use of BMA for blue growth and blue biotech applications** (see 5 for more details).

(5) Finally, BIO-Tide partners will continue with their efforts to **inform and involve the public at large** by raising awareness about the importance of microbial diversity for the functioning of coastal ecosystems, though various outreach activities (see 5 for examples).

5. Stakeholder engagement in the project

5.1 Before the project's start

As outlined in the project application, we closely interacted with stakeholders during the writing and framing of the project proposal (all partners). Before the writing of the project, we made a list of potential stakeholders, including private companies, policy makers, and public and academic organisations/institutions. These were contacted and depending of level of engagement, interested stakeholders were categorized into a final list of collaborating, involved, consulted and informed stakeholders. These were subsequently included in the project proposal (see proposal for list). In addition, we thought about ways to reach out to our main stakeholder, which is the public at large, in order to raise awareness amongst the general public that the microbial organisms inhabiting tidal sediments are hugely important, just like their counterparts in the plankton.

For the private stakeholders, focus was mainly on companies operating in the Baie of Bourgneuf area (e.g. Benth'ostrea). This area was the site of our first joint field campaign (2017), but was also the main focus area of additional seasonal campaigns and the remote sensing work. This area is highly relevant for BIO-Tide as it is intensively used for oyster farming, and hence presents interesting questions regarding reconciliation of potentially conflicting interests by different stakeholder groups (oyster farmers, tourism, nature conservation).

At the beginning of the project the research teams of P4 and P5 met with several stakeholders (including BIO-LITTORAL, Benth'ostrea, ONEMA and Marinarium Concarneau) in order to further delineate their expectations regarding interactions within the BIO-Tide project. It was during one of these meetings that Benth'ostrea asked us to explore the use of benthic diatoms for sea urchin farming.

5.2 During the project

During the project, some stakeholders inevitably dropped out, while new stakeholders (including a biotech start-up company) were identified and included. Below, the engagement with and contributions of the stakeholders are listed.

Engagement with private stakeholders

BIO-LITTORAL (http://www.bio-littoral.fr/) participated to the first field campaign (La Coupelasse) and delivered macrobenthos data for the project (WP1). These data together with the data collected by the partner research teams will enrich their biodiversity database for the Region Pays de la Loire. This database is then used in their environmental impact studies. BIO-LITTORAL also actively participated to some project meetings and also attended the 2015-16 Call Projects' Kick Off meeting and The BiodivERsa Research and Innovation Workshop in Brussels (April 2017).

Together with BENTH'OSTREA (http://www.benthostreaprod.fr/), P5 set up a very fruitful collaboration in the field of sea urchin aquaculture (WP4). While initially collaboration was going to be focused on oyster cultivation, it soon appeared they were very interested in exploring the potential of BMA biodiversity to sea urchin cultivation. Sea urchin aquaculture is an emerging market in Europe but little is known about the role of BMA diversity in sea urchin feeding. The experiments showed that recruitment of the sea urchin *Paracentrotus lividus* can successfully be stimulated using diatom-based biofilms. It was shown that biofilms of the diatom *Nitzschia laevis* were effective in induding sea urchin metamorphosis, and form a practical and safe alternative to the use of natural biofilms for aquaculture farmers, as it overcomes potential contamination. These results were confirmed by follow-up experiments in the framework of a MSc thesis. Interactions with this stakeholder are still ongoing, but have been stalled because of the COVID-19 pandemic.

A promising new collaboration was started up between P5 and the French biotech startup SYNOXIS ALGAE (https://www.synoxis-algae.com)(WP4,6). This company designs and builds new generation photobioreactors to facilitate microalgae cultivation for professionals. Photobioreactors are designed to grow microalgae in suspension (mainly planktonic species), but are often not suitable to grow benthic microalgae. The latter however represent an underexplored and promising new source for interesting biomolecules and feed for aquaculture (cf. results of interaction with Benth'Ostrea). A meeting was therefore set up with Synoxis Algae (09/2020) to explore the possibility of adapting and optimizing one of their photobioreactor systems for the cultivation of benthic diatom species. The meeting was very productive and a work roadmap was developed to combine the scientific expertise on benthic diatom ecophysiology of the BIO-Tide partner with their know-how to develop a porous substrate photobioreactor (PSBR)(based on photo-bioreactor model "Nano" from Synoxis Algae) into a version that can be used to optimize biomass yield of BMA in biofilms. This collaboration will be further developed in the framework of the PhD research of Mary Arnaldo (funded by the French Research Ministry, 2019-2022).

Another collaboration was started up between P4 & P5 and private and public stakeholders in the frame of the Holofarm project (European Maritime and Fisheries Fund, EMFF). This project is aimed at launching sea cucumber farming as a new aquaculture industry, based on successfully breeding and raising native sea cucumbers. It is focused on understanding the target species better and on perfecting breeding and production cycles, from hatchery through to fattening. The project brings together academic, public and private stakeholders such as the AQUA B company (specialised in the processing and preservation of fish, crustaceans and molluscs, http://marinoe.fr/) and the Regional Shellfish Farming Committee of North Brittany. A preliminary study performed by P4 revealed that wild *Holothuria forskali* appears to mainly feed on BMA (diatoms) instead of seaweeds [David et al., Aquacult. Nutrit. 26:1568–1583 (2020)], underscoring the importance of BMA biofilms for this emerging aquaculture sector. A follow up project (Holotrack, 2021-2023, also involving AQUA B) was started with funding from the local development branch (DLAL) of EMFF. On the basis of the knowledge gained in the BIO-TIDE project, P4 will further study the trophic ecology of sea cucumbers and develop a traceability method to differentiate between holothurians from wild populations vs aquaculture.

Engagement with public stakeholders

For data management, all partners closely collaborated with the Flanders Marine Institute (VLIZ, https://www.vliz.be/). More details are given in section 7.5.

A new collaboration was set up with the Royal Netherlands Institute for Sea Research (NIOZ) ate Yerseke (The Netherlands, www.nioz.nl). NIOZ scientists participated in the second field campaign (Westerschelde estuary, 2018, WP1, P1,3-6) and very generously gave access to their lab and storage facilities during this campaign.

The Nantes University Observatory of Universe Sciences (OSUNA: https://osuna.univ-nantes.fr/) was included as a collaborative stakeholder for remote sensing approaches, especially for hyperspectral imaging. This Observatory is the owner of the hyperspectral camera used during BIO-Tide project (HySpex Mjolnir VS-620), and supported P5 by co-funding the project HypEddy (see below).

The Brittany region was identified as a new stakeholder in the frame of the SAD program which aims to improve the attractiveness of the Brittany territory through support for incoming foreign researchers. A cofunding project was settled between the Brittany region and the BIO-Tide project to help funding the GC-MS system and by funding a one-year postdoctoral researcher who developed the metabolomics part in the BIO-Tide project (P4, WP1).

In 2019, the Finistère local council (CG29) was identified as a new public stakeholder. CG29 identified P4 as an emerging research entity in the Finistère locality and provided additional support in the frame of the APRE program which aims at helping new local research themes. A new cofunding project was thus settled between CG29 (APRE program) and the Brittany region (SAD program) to provide support for human resources in a follow-up project on marine MPB biofilms metabolomics. This Project (BioMar) will start in 2021 for a minimum of 18 months and aims to explore the latest advances in the field of photonics to lead to the destruction of marine biofilms (as an antifouling approach) while ensuring minimum ecological impact. The project is focused on the analysis of the fine composition of the biofilm in terms of the metabolites involved in the structuring of the exopolymeric matrix. These analyses will be carried out by the mass spectrometry platform at the Concarneau marine station cofinanced by the BIO-TIDE project.

P1 participated in the 'Sediment & Ecology' workshop in Antwerp (BE, 21 June 2018). This workshop, organized by the Department of Mobility & Public Works (Flanders, BE), brought together scientists, public servants and specialised private consultancies to exchange knowledge and discuss the relationship between sediment regimes and ecology in the Schelde estuary. More specifically, the Flemish government wants to apply this knowledge in the framework of the CP ECA ('complex project extra containerbehandelingscapaciteit Antwerpen'), aimed at evaluating what the impact would be of increasing container processing capacity in the harbour of Antwerp. One of the main knowledge gaps concerns the role of tidal flats in the sediment dynamics in the Schelde estuary, and thus relates to one of the main services of tidal flats identified in the BIO-Tide project, i.e. sediment stabilization by microbial biofilms. Following up on this theme, P1 (in collaboration with University of Antwerp, BE) successfully applied for a doctoral grant (FWO Flanders) on the topic 'The importance of microphytobenthos in sustainable ecosystem management: benthic primary production and sediment stability in estuaries', which also involves a collaboration with P5 and stakeholder NIOZ.

A delegation from the Merinov Center for Applied Research (Canada, https://www.merinov.ca/en/) visited the Concarneau Marine Station in April 2018 (P4). Merinov is Canada's largest integrated centre for applied research in fishing, aquaculture, and the processing and development of aquatic products. In 2018, Merinov sent 2 delegates to Brittany to improve research collaborations. P4 was contacted to explore possible ways to use BIO-TIDE results for industrial applications for marine biotechnology. Metabolomic measurements from the second field campaign could be interesting in this context and further discussions and meetings will be scheduled with P4 and other BIO-TIDE partners if the results are promising.

Finally, several partners (P1,3) serve on **policy advisory boards**. T. Moens (P3) is a member of the Committee Monitoring Westerschelde, an independent scientific organ which provides advice on the monitoring strategy related to the dredging and other major anthropogenic activities in the Schelde Estuary and their impact on ecological status. The monitoring of the status and functioning of intertidal flats is a major point of attention in the different advisory reports produced by the Committee. In addition, since 2020, he is also a member of the multidisciplinary technical-scientific committee 'A vision on the coast', a policy-advising committee which has been established to assess different scenarios on the future of Belgian coastal ‘landscapes’ under future climate scenarios. B. Jesus (P5) was a scientific coordinator of the workshop “Land-sea continuum and interfaces”, which was organized in 2020 by the CNRS-INSU (French Institute for Universe Sciences) to define scientific, technological and organizational priorities for the next 10 years. The final synthesis meeting (https://premc.org/prospective-insu/) has identified the better knowledge of social ecosystems constituting the costal zone, including mudflats as a national priority.

Engagement with academic stakeholders

Several new academic stakeholders were involved in the project: Prof. E. Geslin (Univ. Angers, FR, interactions foraminifera - BMA), Prof. M. Kühl (Univ. Copenhagen, DK, photophysiology BMA), Prof. V. Cnudde (Research Unit Pore Scale Processes in Geomaterials (PProGRess), Ghent University, BE, bioturbation nematodes), Prof. S. Bouillon (Leuven University, BE, SIP benthic food webs) and Prof. T. Lueders (Institute of Groundwater Ecology, Helmholtz Zentrum, Munich, D, RNA-SIP analyses).

5.3 Foreseen after the project’s end

The BIO-Tide project has been a **key enabler** in securing additional funding for numerous follow-up projects and collaborations, including several initiatives in the **Blue Growth** sector. Some of these have been approved, while others are still in the preparation or evaluation stage. Below a selection of the most important projects and ongoing initiatives are listed. The partners involved and the WPs that the follow-up project is derived from is indicated between brackets.

Scientific projects

- FWO-Flanders research project - Substrate-mediated interactions between diatoms and bacteria in marine biofilms (2020-2023) - The objective of this project is to investigate to what degree species-specific associations between diatoms and bacteria in marine biofilms are mediated by specialized interactions related to EPS production and utilization, and how this affects coexistence and hence biofilm diversity (P1)(WP2).
- FWO-Flanders research project ‘Contribution of chemoautotrophic and chemosynthetic organic matter production pathways to freshwater and estuarine benthic invertebrate communities (2018-2021) – This project, built on the results of the BIO-Tide 2017 SIP experiment, uses SIP approaches to investigate both photo- and chemoautotrophic pathways in benthic ecosystems (P3)(WP1,3)
- EMBRC - Hercules Foundation ESFRI (FWO-Flanders) – A Marine Research Platform for access to and the study of Marine Species – Flanders Regional EMBRC (European Marine Biological Resource Centre) node for Marine Biological Resources (2021-2024)(P1, WP1,2)
- FWO Int. Coord. Act - INTERTIDE: INTERcomparison of TIDal Estuaries in NW Europe (2018-2021)(P1, WP 1)
- FWO-SBO PhD scholarship (D. Bas) - The importance of Microphytobenthos in sustainable ecosystem management: benthic primary production and sediment stability in estuaries (2020-2023) - This project is aimed at quantifying BMA primary production using remote sensing tools, relating this to sediment stabilization potential and implement these functions in a calculation tool for ecosystem functioning in the Schelde estuary (P1, WP6).
- CNES-TOSCZA (French Spatial Agency) - HypEddy - Cartographie de la Production Primaire du microphytobenthos des vasières intertidales par Télédétection Hyperspectrale: couplage Réflectance / Eddy Covariance atmosphérique project (2019-2021) - The objective of this project is to link hyperspectral imagery acquired at varied scales by drone and by plane and C fluxes measured by Eddy-Covariance (EC)(P5, WP6).
- CSC (China Scholarship Council) PhD scolarship (M. Zhang) - Cartographie de la production primaire brute du microphytobenthos des vasières littorales en utilisant un couplage de la télédétection hyperspectrale avec des mesures de flux de carbone à l’interface sédiment/air (2019-2022)(P5, WP6)

Blue Growth projects

Below some projects and ongoing initiatives highlighted.

- FEAMP/Brittany region, Holotrack - traceability method for the holothurian *Holothuria forskali* (2020-2022)(P4, WP4)
- Brittany region, BioMar - knowing marine biofilms: using photonics to bring down the microbial fortress (2020-2022)(P4, WP2)
- BIO-Tide results supported the hypothesis that BMA biodiversity is also likely to have an effect on biofilm optical patterns, and consequentially on the interpretation of remote sensing imagery. This led to the participation of P5 in the project “Bio-inspired and Bionic materials for Enhanced Photosynthesis” (BEEP), which is an interdisciplinary research training network funded by the EU H2020 program (https://www.ch.cam.ac.uk/beep/home). This an ongoing project where the role of P5 is to determine how BMA biofilm diversity affects biofilm optical properties and investigate how BMA diversity can be used to improve existing biophotonic materials.
- SMIDAP (Regional organization for fishing and aquaculture development) Biofilm project - Biofilm culturing and ecophysiological approach for lipid production optimizing of benthic diatom strains from Loire ecosystems - The objective of this project is to optimize culture conditions for benthic marine diatoms identified during the BIO-Tide and AMI (Atlantic MicroAlgae) projects as lipid producers (P5, WP4).
- Blue Economy CRC biofouling scoping study (Australia) - Role of microbial biofilms in conditioning nets for invertebrate biofouling in offshore fish industries. Proposal submitted (P1, WP2).
- Global Inter-Korean Marine Project (Ghent University Global Campus, Incheon, South Korea; Korean Ministry of Science and ICT) – This projects aims at studying and valorizing BMA diversity from Korean tidal flats. Proposal submitted (P1, WP1,2,4).
- Intercluster cSBO project – Enzymares - Enzyme prediction toolbox. This projects aims at exploiting BMA diversity (among other marine microbes) to mine for novel marine enzymes. Proposal in preparation (P1, WP4).

6. Dissemination of results

6.1 List of scientific publications

See separate excel list and electronic versions of the manuscripts. Manuscript numbers, used for referencing throughout the report, are listed in this table.

M1	**D’Hondt, A.-S., Stock, W., Blommaert, L., Moens, T. & Sabbe, K.** 2018. Nematodes stimulate biomass accumulation in a multispecies diatom biofilm. Marine Environmental Research 140 : 78-89.
M2	**Méléder, V., Jesus, B., Barnett, A., Barillé, L.**, Lavaud, J. 2018. Microphytobenthos primary production estimated by hyperspectral reflectance. PLoS ONE 13(5): e0197093.
M3	Koedooder, C., **Stock, W.,** Mangelinckx, S., Willems, A., Vyverman W. **Sabbe K**. 2019. Diatom-bacteria interactions modulate the composition and productivity of benthic diatom biofilms. Frontiers in Microbiology 10: 1255.
M4	Savelli, R.**,** Bertin, X., Orvain, F., **Gernez, P.**, Dale, A., Coulombier, T., Pineau, P., Lachaussée, N., Polsenaere, P., Dupuy, C. & Le Fouest, V. 2019. Impact of chronic and massive resuspension mechanisms on the microphytobenthos dynamics in a temperate intertidal mudflat. Journal of Geophysical Research: Biogeosciences, 124, 3752-3777.
M5	**Stock, W.**, **Blommaert, L.,** De Troch, M., Mangelinckx, S., Willems, A., Vyverman, W. & **Sabbe, K**. 2019. Host specificity in diatom-bacteria interactions alleviates antagonistic effects. FEMS Microbial Ecology 95, 2019, fz171.
M6	**Stock, W., Blommaert, L., Daveloose, I.**, Vyverman, W. & **Sabbe, K.** 2019. Assessing the suitability of Imaging-PAM fluorometry for monitoring growth of benthic diatoms. Journal of Experimental Marine Biology and Ecology 513: 35-41.

M7	**Wu, X.**, Bezerra, T.G., Van Gansbeke, D. & **Moens, T.** 2019. Natural stable isotopes and fatty acid profiles of estuarine tidal flat nematodes reveal very limited niche overlap among co-occurring species. PeerJ 7: e7864.
M8	**Stratmann T**., **Soetaert K.**, Wei C.-L., Lin Y.-S., & van Oevelen D. 2020. The SCOC database, a large, open, and global database with sediment community oxygen consumption rates. Scientific Data 6: 242.
M9	**Castilla-Gavilá, M.**, Meshi, R., Turpin, V., Decottignies, P., **Cognie, B.**(2020) Sea urchin recruitment: Effect of diatom based biofilms on Paracentrotus lividus competent larvae Aquaculture 515: 734559.
M10	**Cointet, E.,** Wielgosz-Collin, G., Bougaran, G., Rabesaotra, V., Gonçalves, O., & **Méléder, V.** 2019. Effects of light and nitrogen availability on photosynthetic efficiency and fatty acid content of three original benthic diatom strains. PloS ONE, 14(11), e0224701.
M11	**Gaubert-Boussarie, J.**, Prado, S. & **Hubas, C.** 2019. An untargeted metabolomic approach for microphytobenthic biofilms in intertidal mudflats. Frontiers in Marine Science 7: 250.
M12	**Méléder, V.,** Savelli, R., **Barnett, A.,** Polsenaere, P., **Gernez, P.** Cugier, P., Lerouxel, A., Le Bris, A., Dupuy, C., Le Fouest, V. & Lavaud, J. 2020. Mapping the Intertidal Microphytobenthos Gross Primary Production Part I: Coupling Multispectral Remote Sensing and Physical Modeling. Frontiers in Marine Science 7: 520.
M13	**Prins, A.,** Déléris, P., **Hubas, C., Jesus, B.** 2020. Effect of Light Intensity and Light Quality on Diatom Behavioral and Physiological Photoprotection. Frontiers in Marine Science 7: 203.
M14	Savelli, R., **Méléder, V.,** Cugier, P., Polsenaere, P., Dupuy, C., Lavaud, J., **Barnett, A.** & Le Fouest, V. 2020. Mapping the intertidal microphytobenthos Gross Primary Production. Part II: Merging remote sensing and physical-biological coupled modelling. Frontiers in Marine Science 7: 521.
M15	David, F, **Hubas, C,** Laguerre, H, et al. Food sources, digestive efficiency and resource allocation in the sea cucumber Holothuria forskali (Echinodermata: Holothuroidea): Insights from pigments and fatty acids. Aquacult Nutr. 2020; 26: 1568– 1583

6.2. Dissemination of results to scientists and scientific organisations (1-page max)

Data were exchanged with the academic stakeholders (cf. 5.2). There was no significant interaction with other BiodivERsa projects apart from the general BiodivERsa meetings (see below).

Presentations

- Sabbe, K. et al. ‘Reciprocal interactions shape the structure and functioning of diatom-bacteria consortia in marine biofilms’, 11th Int. Phyc. Congr., 08/2017, Szczecin, Poland (P1).
- Jesus, B. et al., ‘Single-cell analysis of species-specific photo-regulation and photo-acclimation status of microphytobenthic diatoms’, AQUAFLUO II - Chlorophyll Fluorescence in Aquatic Sciences Meeting, 12/ 2017, Sydney, AUST.
- Méléder, V. et al. ‘Radiometry vs. fluorometry: the use of hyperspectral reflectance to retrieve photosynthetic parameters from algal biofilms’. AQUAFLUO II - Chlorophyll Fluorescence in Aquatic Sciences Meeting, 12/2017, Sydney, AUST (P5).
- Moens, T. et al. Inter-and intraspecific competition influence the resource utilization and niche width of closely related nematode species. 17th Int. Meiof. Conf., Evora, Portugal, July 2019 (P3).
- Moens, T. & X. Wu. Natural stable isotope ratios and fatty acid profiles of estuarine tidal flat nematodes reveal very limited niche overlap among co-occurring species and a high prominence of omnivory. 17th Int. Meiof. Conf., Evora, Portugal, July 2019 (P3).
- Vafeiadou, A.-M., et al. Microbiome partitioning of coexisting nematode species in shared microhabitats: insights of a metagenetic analysis. 17th Int. Meiof. Conf., Evora, Portugal, July 2019 (P3)
- Vafeiadou, A.-M. & T. Moens. Potential drivers of species coexistence of marine nematodes. VLIZ Marine Science Day. Bredene, Belgium, 13 March 2019 (P3)
- Wu, X. et al. Characterization of marine nematode associated microbiomes by high-throughput sequencing. 17th Int. Meiof. Conf., Evora, Portugal, July 2019 (P3)

- Stock, W. et al. The complex interplay between environment, diatoms and bacteria shapes the structure and functioning of biofilms in intertidal mudflats. 3^{rd} Microphytobenthic Biofilm Symposium, 01/ 2018, Southend, UK (P1-5), awarded best student presentation.
- Echappé, C. et al. 'A life-size experiment coupled to satellite time series reveals living oyster reefs promoting impact on microphytobenthos development', 3^{rd} Microphytobenthic Biofilm Symposium, 01/2018, Southend, UK (P1,5)
- Jesus, B. et al. 'Single-cell analysis of species-specific photo-regulation and photo-acclimation status of microphytobenthic diatoms', 3^{rd} Microphytobenthic Biofilm Symposium, 01/ 2018, Southend, UK (P5).
- Méléder, V. et al. 'Radiometry vs. fluorometry: the use of hyperspectral reflectance to retrieve photosynthetic parameters from algal biofilms'. 3^{rd} Microphytobenthic Biofilm Symposium, 01/ 2018, Southend, UK (P5).
- Stock, W. et al., 'The BIO-Tide project: The role of microbial biodiversity in the functioning of marine tidal flat sediments', VLIZ Marine Science Day, 03/2018, Bruges, BE (P1-5), awarded with best oral pre-doc award.
- Sabbe, K. et al. 'BIO-Tide: An international collaboration to tackle the complexity of marine tidal flats' Eur. Mar. Biol. Symp., 09/2018, Ostend, BE (P1-5)
- Sabbe, K. et al. 'Microalgal biodiversity from Korean tidal flats: inventory, importance and valorization'. South and North Korea Marine Global Project, 03/2019, Incheon, South Korea.
- Savelli, R. et al. 'Modeling of chronic and massive resuspension mechanisms of microphytobenthos on a temperate intertidal mudflat', ASLO Aq. Sci. Meet., 03/2019, San Juan, Puerto Rico (P5)
- Zhang, M. et al. 'Coupling PAM-fluorometery, C-fluxes and reflectance to estimate microphytobenthos primary production by hyperspectral remote-sensing.' 7^{th} Eur. Phyc. Congr., 08/2019, August, Zagreb, Croatia (P5)
- Sabbe, K. et al. 'Ecological and functional implications of host-specificity in diatom-bacteria associations'. 16th Symp. Aquat. Microb. Ecol., 09/2019, Postdam, D (P1).
- Sabbe, K. et al. 'Implications écologiques et fonctionnelles de la spécificité d'hôte dans les associations de diatomées et de bactéries'. Colloque ADLaF, 09/2019, Metz, FR (P1).
- Stratmann T, et al. 'Carbon processing differs between sandy and silty sites at Bourgneuf Bay (France)'. Ann. Ecol. Meeting, 02/2020, Lunteren, NL (P1,3-5)

Posters

- Stock et al. 'Tracing carbon in marine phototrophic biofilms'. 7^{th} Eur. Phyc. Congr., 08/2019, August, Zagreb, Croatia (P1-3).
- Castilla-Gavilán et al. 'Sea urchin recruitment: the effect of diatom based biofilms on *Paracentrotus lividus* competent larvae'. EAS, 2019 Berlin (P5)

Partner 1, 5 and stakeholder BIO-LITTORAL actively participated to the 2015-16 Call Projects' Kick Off meeting and The BiodivERsa Research and Innovation Workshop in Brussels (April 2017). Partner 1 actively participated to the final conference of the research projects funded through the BiodivERsa 2015-2016 call and the Strategic Workshop on a possible Biodiversity Partnership and the co-organized by BiodivERsA and the European Commission (DG R&I, DG ENV) in Brussels (Nov 2019).

6.3 List of dissemination activities with stakeholders

Dissemination activities with all categories of stakeholders are described in detail in section 5. Throughout the project lifetime, we closely interacted with specific stakeholders via project and dedicated meetings and active collaborations in the field and/or lab.

6.4 Dissemination of results to stakeholders (1-page max)

Dissemination of results to stakeholders is described in section 5. Information was transferred to the stakeholders via meetins and sharing of data.

BIO-Tide partners have been very active in various **outreach** initiatives **to the general public**. V. Méléder (P5) closely interacted with artists of the Atelier Lucile Viaud (https://atelierlucileviaud.com/) who made sculptures of marine diatoms from marine glass, the 'Glaz', deriving its particularity from the use of diatoms as a source of silica (https://atelierlucileviaud.com/verre-marin-glaz). The sculptures have been exhibited at the MerXXL event (July 2019, Nantes), at the "Nuit Blanche des Chercheurs" (February 2020, Nantes), at the MIRA gallery (March 2020, Nantes) and at the Médiathèque of Yffiniac (Festival Baie des Sciences, October-November 2020, Yffiniac). This exhibition aims to raising awareness of micobial diversity, and more specifically diatoms, by unveiling the beauty of the organisms to the public at large (for catalogue with BIO-Tide acknowledgment: https://atelierlucileviaud.com/wp-content/uploads/2020/10/ALV_OSTRACO_Diatomees-Septembre2020_paysage.pdf). V. Méléder (P5) also gave an interview on the topic of tidal flat BMA (diatoms) on the web-radio "Labo des Savoirs" (https://youtu.be/XZUjNM2fWLY) in April 2020, and again during a 45 min radio program in November 2020 (https://labodessavoirs.fr/emissions-du-labo/diatomees-de-la-vase-au-verre/).

W. Stock and others (P1) participated in an exhibition and demonstration stand on the importance of algae (and especially how they use light) at the Ghent Light Festival (Feb. 2018); in a period of three days, > 5000 visitors visited the stand. W. Stock (P1) also presented microbial life in mudflats during a selected talk at the TEDxGhent PhD Contest for a 100-150 students (Oct. 2018). K. Sabbe and W. Stock (P1) hosted an exhibition stand at the Wooow festival ('Day of Science', http://www.wooowfestival.be/), held in Ghent (Nov. 2019). This annual science festival is mainly aimed at families. Fresh sediment cores were shown to show the visitors the marine BMA biofilms and zoobenthic organisms. In addition visitors could look down microscopes at live microalgae.

K. Sabbe (P1) and T. Moens (P3) gave scientific advice on the content and design of the new exhibition halls (opening Nov. 2020) on the ocean and its inhabitants (and especially its microbial inhabitants) at the House of Kina (https://dewereldvankina.stad.gent/), a natural history museum for children in Ghent (Be).

C. Hubas (P4) participated to the 2018 Océanopolis scientific conference program on the importance of meiofauna in intertidal sediments. Océanopolis has become a very unique Ocean Discovery Park, combining scientific approach with spectacular settings in its three thematic pavilions: Polar, Temperate and Tropical, and with an average number of 450,000 visitors every year. C. Hubas (P4) published an online article on The Conversation (https://theconversation.com/quand-les-marees-vertes-virent-au-pourpre-144765). He is also negotiating with the Marinarium (public aquarium of the Concarneau marine station) to exhibit results of the BIO-Tide projects, in the form of appealing images of microalgae, field and lab work, accompanied by texts that explain the importance of microbial diversity in tidal flats to the general public. P5 is also exploring whether the BIO-TIDE project can be presented during a special session during the Concarneau rendez-vous ('where industry meets science!') which each year focuses on a special topic related to biotechnology.

Most BIO-Tide partners are involved in academic teaching, creating opportunities to share BIO-Tide results with students, and to involve them in BIO-Tide related research activities (in stages, practical courses, BSc and MSc theses). The BIOTIDE programme has also been mentioned in a number of Seminars and in Teaching programmes at the University of St Andrews and is aligned with the aims of the Marine Alliance for Science and Technology for Scotland (MASTS), encompassing 18 marine organisations across Scotland (P6), and in French schools and universities, but also during public events as "La Mer XXL" (July 2019, Nantes) as a sponsor of the conference "Intertidal mudflats: a less and less secret garden" given by V. Méléder (P5).

The BIOTIDE work aligns closely with the biodiversity and ecosystem function theme as well as contributing to national project such as the C-SIDE (Carbon Storage in Intertidal Environments (C-SIDE). Ref NE/R010846/1) and the newly funded NERC INSITE Programme (NERC INSITE award NE/T010800/1)(P6). Joint dissemination and outreach on the issue of biodiversity ecosystem function and ecosystem service protection and delivery will be supported by these programme and further communicated with Government agencies such as Marine Scotland Science (MSS), The Joint Nature Conservation Committee (JNCC) and Nature Scot, among others.

In collaboration with the BCCM-DCG diatom collection (http://bccm.belspo.be/about-us/bccm-dcg, hosted in the lab of P1), cheap teaching sets were developed containing strains (including tidal flat diatoms), media and plastic ware for (Belgian) schools and universities to learn students how to start to grow diatoms.

7 Global Impact assessment indicators

7.1 Impact statement

The impact of the BIO-Tide project has been highly significant:

1. The project delivered new knowlegde on the relationship between biodiversity and ecosystem functioning in tidal flat sediments. These new insights revealed that microbial diversity matters for ecosystem functioning, and that this relationship is driven by trophic interactions but also by other as yet not fully characterized interactions between bacteria, benthic microalgae, meio- and macrobenthos. While numerous scientific publications have been produced, and the results have been presented at (inter)national conferences, many manuscripts, especially multi-partner ones, have been delayed because of lab lockdowns and lack of in person communication related to the COVID-19 pandemic.
2. Through involvement of several partners in coastal management and policy platforms and interaction with public stakeholders (see 5.2), we ensure that the new knowledge resulting from the BIO-Tide project will feed into ICZM strategies.
3. We closely collaborated with selected private stakeholders to apply and transfer the BIO-Tide knowlegde outside the sphere of pure fundamental science. Successful collaborations with private stakeholders in the aquaculture field include promising new avenues in sea urchin and sea cucumber culture, and development of photobioreactors for growing benthic microalgae. We further explored benthic microalgal biodiversity for the production of commercially interesting lipids.
4. We developed a new approach for inventorying the diversity of little-known biofilm metabolites which include metabolites of applied interest (aquaculture). In addition, we developed a new remote sensing approach to map tidal biofilm primary production at a wide range of spatial scales. This important breakthrough will allow to better constrain primary production estimates of these systems, and assess their contribution to coastal ecosystem integrity and functioning. These results will be of great value for both private and public stakeholders involved in ICZM.
5. We have been involved in numerous outreach and educational activities (see 6.4) to increase awareness of the importance of microbial organisms for the proper functioning of coastal ecosystems. All partners recognize the importance of such activities for garnering public support for science.
6. As explained in 5.3, the BIO-Tide project has been a crucial enabler for the project partners to engage in further fundamental and applied collaborations and projects with existing and new partners and stakeholders, especially in the Blue Growth sector. The insights, collaborations and prestige generated by the project facilitated successful new applications with a wide range of funding agencies.

7.2 Synthetic figures for the project publications (including interactions with stakeholders)

During the lifetime of the BIO-Tide project, 15 papers were published. One paper is submitted and 19 papers are in preparation (listed in the publication list template, but not taken into account in the following table). In the Table below, a list of the journals with impact factor (see publication list template).

Scientific journal	Number	Impact factor
Scientific Data	1	5.929
Frontiers in Microbiology	1	4.236

FEMS Microbial Ecology	1	3.675
Frontiers in Marine Science	4	3.661
Journal of Geophysical Research: Biogeosciences	1	3.480
Marine Environmental Research	1	3.445
Aquaculture	1	3.225
PloS ONE	2	2.776
PeerJ	1	2.379
Journal of Experimental Marine Biology and Ecology	1	2.247
Aquaculture nutrition	1	2.231

International dimension and multi-partnership for publications

For a number of reasons (see 4.5), the preparation of multi-partner papers is still ongoing. Most of these will be submitted in the course of 2021.

		Number of publications
Multi-partner publications	**Peer-reviewed journals**	2
	Books or chapters in books	3
	Communications (conferences)	7
Single-partner publications	**Peer-reviewed journals**	13
	Books or chapters in books	1
	Communications (conferences)	13
Outreach initiatives including interactions with stakeholders	**Popularization articles**	2
	Popularization conferences	See 6.4
	Others	

7.3. Other scientific outputs

	Number, years and comments (Actual or likely outputs)
International patents obtained	None
International patents pending	None
National patents obtained	None
National patents pending	None
Operating licences (obtained / transferred)	None
Software and any other prototype	None
Company creations or spin-offs	None
New collaborative projects	See section 5.3.
Scientific symposiums	None organized
Others (please specify)	Depositions of strains in BMA diatom strains the BCCM-DCG collection (https://bccm.belspo.be/about-us/bccm-dcg)

7.4. Assessment and follow-up of personnel recruited on fixed-term contracts (excluding interns)

Identification			Before recruitment for the project			Recruitment for the project				After the project			
Surname and first name	Sex M/F	E-mail address	Last diploma obtained at time of recruitment	Country of studies	Prior professional experience, including post-docs (years)	Partner who hired the person (Organisation and Country)	Position in the project (1)	Duration of missions (months) (2)	End date of mission on project	Professional future (3)	Type of employer (4)	Type of employment (5)	Promotion of professional experience (6)
Stock Willem	M	Willem.stock@ugent.be	MSc	Belgium	-	Ghent University-Belgium	Doctoral student	12	01-12/2019	Post-doctoral position	teaching and public research	researcher	Yes
Margarita Bogorad	F	Margarita.bogorad@ugent.be	MSc	Israel/Belgium	-	Ghent University-Belgium	Doctoral student	4	09-12/2019	PhD position	teaching and public research	researcher	Yes
Daveloose Ilse	F	Ilse.daveloose@ugent.be	BSc	Belgium	-	Ghent University-Belgium	Technician	4	01-04/2020	Fixed-term contract		technician	Yes
Stock Willem	M	Willem.stock@ugent.be	PhD	Belgium	-	Ghent University-Belgium	Post-doctoral position	2	01-02/2020	Post-doctoral position	teaching and public research	researcher	Yes
Margarita Bogorad	F	Margarita.bogorad@ugent.be	MSc	Israel/Belgium	-	Ghent University-Belgium	Doctoral student	5	01-05/2020	PhD position	teaching and public research	researcher	Yes
D'Hondt An-Sofie	F	Ansofie.dhondt@ugent.be	MSc	Belgium	3 years prior to BIO-Tide contract	Ghent University - Belgium	Scientific staff, PhD student	9	31-07/2018	Re-oriented to studies in medicine	student	none	No
Wu Xiuqin	F	Xiuqin.wu@ugent.be	MSc	China	6 years prior to contract on BioTide	Ghent University	PhD student	4	31-10/2018	Currently unemployed	none	none	No
Monteiro Luana	F	Luana.dacostamonteiro@ugent.be	MSc	Brasil, Belgium	4.5 years prior to contract on BioTide	Ghent University	PhD student	3	28-02/2019	Fixed-term contract	Teaching and public research	Teaching, mentoring and administration	Yes
Stratmann Tanja	F	Tanja.stratmann@nioz.nl	PhD	Germany, Spain, Denmark,The Netherlands, Belgium	4 years prior to contract on BioTide	Ghent University	postdoc	6.5	30-06/2019	Fixed-term contract	Public research	Researcher	Yes

Dairain, Annabelle	F	Annabelle.dairain@ugent.be	PhD	France	3 years prior to BioTide contract	Ghent University	postdoc	1.5	31-05-2020	Fixed-term contract	Teaching and public research	Researcher	Yes
Ong Ee Zin	M	ongeezin@gmail.com	MSc	Malaysia, Belgium	3.5 years prior to BioTide contract	Ghent University	PhD student	1	30-06/2019	Currently looking for postdoc	/	/	/
Prins, Antoine	M	antoine.prins@univ-nantes.fr	MSc	France		Nantes University – France	Doctoral student	39	31-05/2020	Finalizing PhD	student	researcher	Yes
Johannes Goessling		johannes.goessling@univ-nantes.fr	PhD	Denmark			Post-doctoral position	3	30/06/2018	Fixed-term contract	Private research	researcher	Yes
Alexandre Barnett	M	alexandre.barnett@univ-nantes.fr	Ph.D	France	Postdoc position in La Rochelle and Galway, teaching position at La Rochelle University, Technician position at La Rochelle, associate professor, La Rochelle University	Nantes University – France	Post-doctoral position	17 (+3)	29/02/2020 (31/05/2020)	Fixed-term contract	Teaching and public research	researcher	Yes
Alexandre Barnett	M	alexandre.barnett@univ-nantes.fr	Ph.D	France	Cf above	Nantes University – France	Post-doctoral position	4.5	30/11/2020	Fixed-term contract	Teaching and public research	researcher	Yes

Data management is coordinated with collaborating stakeholder VLIZ, who hosts the Marine Data Archive (https://mda.vliz.be/). MDA is one of the repositories recommended by Nature (https://www.nature.com/sdata/policies/repositories#ocean). It can be used both as long-term private archive and open repository: when data are published, or when the BIO-Tide partners decide the data can be disclosed (see below), the data become open access. A file can only be archived if a minimum set of metadata fields are complete, ensuring that the data file is findable and reusable in the future. The MDA ensures safe storage of data. Its policy states that if VLIZ would cease to exist or terminate its data-archiving activities, they will attempt to transfer the data files to a similar organisation that will continue the Agreement with the Data provider under similar conditions if possible. If such a similar organisation can not be found, VLIZ will organize a transfer of all files to each intellectual property right holder or data provider.

In the MDA, the BIO-tide data are stored in a dedicated BIO-Tide folder. Most data generated in the framework of the project (mainly 2017 and 2018 sampling campaigns) have already been uploaded. The data sets will be completed by the end of 2020. In addition, during the project data have been shared via Google Drive.

Upon publication, and at the latest by the end of 2021 (1 year after the project's ending), all data will be made publicly available via online databases (e.g. the NCBI Sequence Reads Archive (SRA, http://www.ncbi.nlm.nih.gov/sra) for omics data, GBIF (following the Darwin Core Standard) and MDA for biodiversity and metadata information).