

Diversity and ecosystem functioning in estuarine intertidal microphytobenthos

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Humanity has become a major biogeochemical force and human domination of the planet has caused massive destruction and fragmentation of natural habitats, eutrophication, climate change, acidification, etc. which caused a dramatic biodiversity loss. This biodiversity crisis raised concern about the consequences of biodiversity loss on ecosystem properties and the goods and services these ecosystems provide to humanity.

The main aim of this PhD thesis was to gain a better understanding of the diversity and functioning of estuarine benthic microalgae communities. Estuarine ecosystems provide some essential ecosystem services to humans such as carbon fixation, sustaining fisheries, coastal protection and nutrient cycling. Although estuaries cover relatively small areas on a global scale, their importance for several biogeochemical processes is disproportionately large. We focused on benthic microalgae inhabiting estuarine sediments; these algae have many essential functions for estuarine and coastal ecosystems: e.g. they are the basis of the food chain for many coastal species, mediate nutrient fluxes and stabilize sediments.

A fundamental requirement to gain insights in biodiversity and ecosystem function relationships is a solid knowledge of the diversity. Assessing diversity is not as trivial as it may seem at first, especially in microbial communities. Traditionally, species have been defined based on morphological properties. A multitude of molecular studies however have demonstrated that morphologically defined species often consist of several genetically distinct lineages. Such genetically distinct but morphologically indistinguishable species are called 'cryptic species', whereas the term 'pseudocryptic species' refers to the occurrence of morphologically similar species which at a closer look do exhibit subtle morphological differences between species. In most cases it is unknown whether (pseudo)cryptic species differ in their ecological preferences or whether they are functionally equivalent. This notion is not only important for questions relating diversity and ecosystem function, but also to understand mechanisms that promote cryptic species coexistence.

In **Chapter 2**, we used sequence data of 3 different genetic markers (ITS rDNA, the 18S rRNA gene and *rbcL*) together with cell wall morphology to show that estuarine populations of the widespread and common benthic diatom *Navicula phyllepta* Kütz. consist of pseudocryptic species. Moreover, we show that these pseudocryptic species differed in their tolerance to low salinities (<5 practical salinity units, psu), which was reflected by their different (but widely overlapping) distribution in the Westerschelde estuary (the Netherlands). Our results show that *N. phyllepta* sensu lato comprises different species with specialized ecophysiological characteristics rather than generalists with a broad adaptability to different environmental conditions.

In **Chapter 3** we further investigated the ecological divergence in closely related (pseudo)cryptic microalgae and assessed pattern in phylogenetic relatedness and ecological niche similarity. We explored if thermal niches can evolve fast on an evolutionary timescale resulting in regular switches between climates or if these niches are instead highly conserved. We composed a set of closely related strains of the globally distributed diatom genus *Cylindrotheca*. We collected strains from a wide range of marine habitats, from coastal plankton to sea ice and intertidal mudflats. We first inferred the evolutionary relationships of these strains using a multi-locus DNA dataset and obtained a well-resolved phylogeny. We then determined temperature preferences of closely related lineages in laboratory experiments. Combining the molecular phylogeny with the thermal niches of lineages revealed a very weak phylogenetic signal in thermal niche characteristics. This indicates that closely related species tend to differ more in thermal niche than expected by a random walk model. This seems to be caused by a combination of adaptive evolution and frequent shifts in environments in related lineages.

Species specific niches and functional traits are not only important for species coexistence and biodiversity-ecosystem function relations (see below), they may also determine how species can cope with disturbance. In **Chapter 4**, we examined the relations between the hydrodynamic

disturbance, biomass, species diversity and functional group turnover in estuarine intertidal microphytobenthos. We used an extensive dataset of benthic diatom assemblages covering a wide range of hydrodynamic disturbance to show that growth form and cell size are important traits to predict the effect of disturbance on functional group species richness. Total microphytobenthos species richness displayed a unimodal relationship with hydrodynamic disturbance and standing stock biomass. Our results are consistent with the intermediate disturbance hypothesis which implicitly assumes that there is a trade-off in functional traits determining competitive ability and disturbance tolerance. These trade-offs lead to maximal diversity when competitive and disturbance tolerant species are able to coexist.

In **Chapter 5** we assessed the effects of species diversity on productivity of intertidal microphytobenthos. We used naturally co-occurring diatom species from intertidal mudflats to experimentally assemble communities with decreasing diversity. Our results demonstrate a highly positive biodiversity effect on production, with mixtures outperforming the most productive component species in more than half of the combinations. These strong positive diversity effects could largely be attributed to facilitation and complementarity effects. In addition, we show mechanistic evidence for facilitation which is partly responsible for enhanced production. We show that a strain of *Cylindrotheca closterium* has the ability to significantly increase its biomass production in response to substances leaked into the culture medium by other diatom species. In these conditions, the species drastically reduced its pigment concentration, which is typical for mixotrophic growth.

In **Chapter 6**, I show that, next to positive, facilitative interactions, direct negative interactions (allelopathy) can strongly influence species composition in microphytobenthos. I show that cell cultures of the marine benthic diatom *N. cf. pellucida* exude metabolites with strong allelopathic effects. All nine competitor species tested were inhibited in their growth and some in their photosynthetic efficiency as well. In addition, I show the occurrence of reciprocal, density dependent allelopathic interactions between *N. pellucida* and two other marine benthic diatom species, *Entomoneis paludosa* and *Stauronella* sp. These results suggest that allelopathic interactions might be common in benthic microalgal communities and may explain small-scale spatial distribution patterns observed in nature.

In **Chapter 7**, I demonstrate the physiological and molecular basis of allelopathic interactions caused by *Nitzschia cf. pellucida*. I show this marine benthic diatom produces chemical cues that cause chloroplast bleaching and a reduced photosynthetic efficiency leading to growth inhibition and massive cell lysis in naturally co-occurring competing microalgae. Using headspace solid phase microextraction (HS-SPME) - GC-MS, I demonstrate that this diatom exudes a diverse mixture of volatile iodinated and brominated metabolites including the new natural products cyanogen bromide (BrCN) and cyanogen iodide (ICN) which exhibits pronounced allelopathic activity. Besides these effective metabolites, this diatom also produces a diverse mixture of mono- di- and trihalomethanes, halogenated acetaldehydes and iodopropane with comparatively lower allelopathic properties. Production of these toxins is light-dependent with a short toxin burst after sunrise. This labile compound thus acts as a short-term signal, leading to daily 'cleaning' events around the algae. We show that the allelopathic effects are H₂O₂ dependent and therefore link BrCN production to haloperoxidase activity. This novel strategy of chemical warfare is a highly effective means of biofilm control. Obviously, such strategy contributes to complex micro-landscapes maintained by interacting species and may boost the small-scale patchy growth habits of biofilm forming species. Our results also provide a potential explanation for the poorly understood role of volatile halocarbons from marine algae which contribute significantly to the atmospheric halocarbon budget.

Overall, our results contribute to the understanding of the species composition and functional organization of complex phototrophic biofilms and its consequences for ecosystem functioning. We suggest that cryptic diversity is widespread among estuarine diatoms and that this diversity is linked with ecophysiological differentiation and habitat partitioning by closely related species. Over large environmental gradients it appears that lineages behave in a conservative fashion, in the sense that they are limited in their distribution to suitable hydrodynamical conditions. At the local scale, strong positive and negative interactions (facilitation, allelopathy) seem to be important in these diatom communities and have a strong influence on the functioning of this ecosystem.