# Shedding light on detritus: Interactions between invertebrates, bacteria and substrates in benthic habitats

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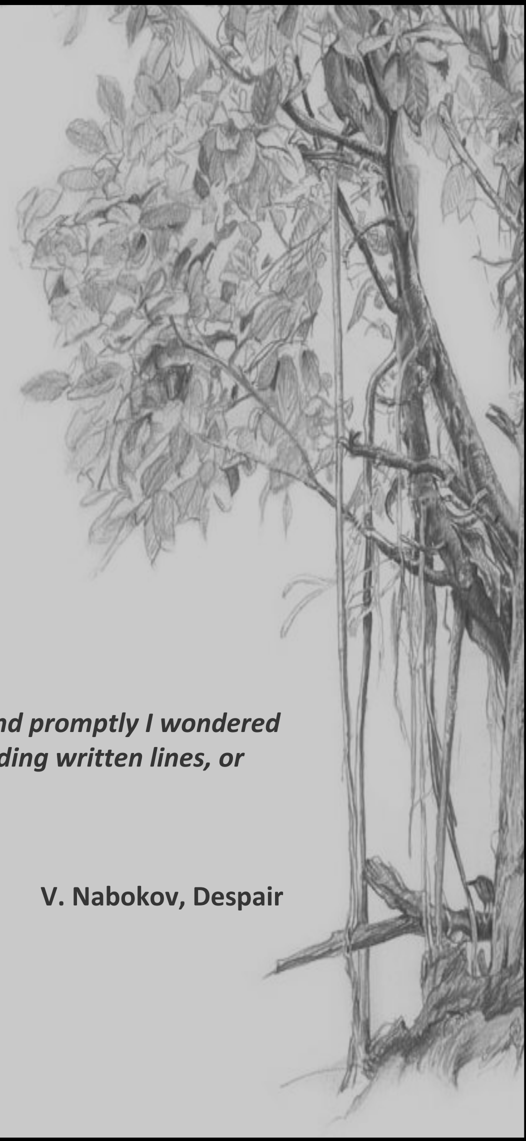

*I began to read, and promptly I wondered whether I was reading written lines, or seeing visions*

**V. Nabokov, Despair**

# ***Chapter 1***

## General introduction

The processing of dead organic matter, also known as detritus, is a central ecosystem process in aquatic habitats driven by detritus feeding organisms that are mostly located at the bottom of water bodies where dead organic matter (OM) accumulates (Webster and Benfield 1986, Graça 2001). These benthic communities are composed of invertebrates, fungi and bacteria, living in, on, or near submerged substrates, and these organisms interact with each other and their substrate in a number of ways. Firstly, facilitative interactions occur among organisms. Although invertebrates are known to consume large amounts of bacterial biomass (Hall and Meyer, 1998), bacteria can also benefit from the presence of invertebrates. For instance, invertebrate litter consumption facilitates the penetration of bacteria into otherwise inaccessible leaf tissue (e.g. De Boer et al., 2005), and sediment reworking by invertebrates can alter sediments by modifying texture, distributing solid particles, and introducing $O_2$ into otherwise anoxic zones (Meysman et al., 2006; Covich et al. 2004, Navel et al. 2010). Invertebrates, on the other hand, can also benefit from the presence of microorganisms. Microbial conditioning of leaf litter increases palatability (Graça, 2001; Canhoto and Graça, 2008), and the production of complementary enzymes or useful carbon products also contributes to a wider use of resources (e.g. Osono, 2007), and detritivorous invertebrates particularly can benefit from this increased diversity in resources (e.g. Lavelle et al., 1998; Hall and Meyer, 1998).

Another important attribute of detritus is that it can vary widely in chemical composition, even within a given plant species (Cornwell et al., 2008; Lecerf and Chauvet, 2008). It is well known that some types of organic matter can easily be utilized (labile carbon), whereas others are nutrient-poor or contain high concentrations of aromatic compounds that are resistant to degradation (recalcitrant carbon) or detrimental to microbial and invertebrate consumers (e.g. Gessner and Chauvet, 2002; Hladyz et al., 2009). Changes in the chemical composition and diversity of OM can therefore entail changes in OM processing by both microorganisms and invertebrates.

Differences in benthic biodiversity and composition of organic matter can thus become visible in the OM processing, and understanding the significance of biodiversity, as well as the mechanistic basis behind effects of biodiversity and OM composition, is essential to assess the consequences of biodiversity change for organic matter processing (Gessner et al., 2010). Biodiversity has long been assessed by characterizing or manipulating species composition, and a number of studies demonstrated that higher levels of biodiversity support higher OM processing rates (Johnsson and Malmqvist 2000; Bell et al., 2005). However, increasing evidence suggest that a functional characterization of communities, i.e. characterizing and sorting species according to the traits that likely affect ecosystem processes (e.g. body size, feeding strategy, locomotion), is likely more valuable in explaining links between biodiversity and OM processing as species with similar functions will unlikely have complementary effects on organic matter processing (e.g. Reiss et *al*., 2009).

Although it is likely that links between benthic biodiversity and OM processing are driven by similar mechanisms across different ecosystem types (forest floors, stream beds, coral reefs) (Gessner et al., 2010), it remains a challenge to identify general drivers of decomposition in distinct benthic detrital food webs.

**Soft sediments and hard substrates**

Two distinct types of substrates, hard substrates and soft sediments, characterize the benthic environment, and these substrates differ in a number of ways. Firstly, they are typically recognized to support different groups of organisms. Shredders, collectors and deposit feeders (e.g. crustaceans, dipterans and oligochaetes), that actively seek and feed on particulate organic matter, abound in soft sediments, while sessile filter feeders (e.g. sponges and bivalves) that feed on particulate and dissolved organic matter dominate hard substrates. Secondly, while mobile invertebrates in soft bottom sediments can move towards more favorable conditions, sessile invertebrates attached to hard substrates are not able to respond to changes in environmental variables, and hence it is likely that abiotic variables more strongly affect hard substrate invertebrate communities as compared to soft bottom sediments, pointing to a potential difference in magnitude of the interactions between the abiotic and biotic components of these distinct substrates. Studying simultaneously a soft bottom sediment system and a hard substrate system would thus provide the unique opportunity to study distinct underexposed aspects of the interaction between the (functional) composition of invertebrate and bacterial communities, organic matter processing and abiotic variables in benthic systems. This thesis therefore focused on the interactions between bacteria, invertebrates and their substrates in two distinct ecosystems: soft bottom sediments and hard bottom substrates, in which gaps in our current understanding of the interactions between abiota, biota and inherent OM processing are identified for both substrates.

*Soft bottom sediments*

Soft bottom sediments harbor a diverse invertebrate community that is composed of species that are widely distributed. Because up to 50% of the particulate organic matter (POM) produced in aquatic ecosystems becomes trapped in subsurface sediments (Herbst, 1980; Metzler and Smock, 1990), the effect of bioturbation on organic matter processing gained increased interest (Mermillod-Blondin and Rosenberg, 2006; Nogaro et al., 2009). Macro-invertebrates alter sediments by modifying texture, distributing solid particles, and introducing $O_2$ into otherwise anoxic zones (Meysman et al., 2006; Covich et al. 2004, Navel et al. 2010). Hence, biological and geochemical components of subsurface sediments might be coupled, suggesting that differences in invertebrate community composition can become visible in ecosystem processes, although this suggestion requires experimental validation.

Species-specific sensitivities to abiotic constraints such as temperature, solar radiation, pH, salinity limit the occurrence of organisms to certain environments (e.g. Bervoets et al., 1996). Likewise, anthropogenic disturbances like eutrophication and environmental pollution may also alter and reduce the diversity of microorganisms and detritivorous invertebrates (e.g. Kiffney et al., 1994; Loayza-Muro et al., 2010; Santos et al. 2012). Moreover, at sub-lethal levels, both natural and human induced abiotic pressure can alter the locomotion and behavior of aquatic invertebrates (Van der Geest et al., 1999; Maltby et al., 2002; Brooks et al., 2009; Bundschuh et al., 2012). It is likely that links between invertebrate bioturbation/feeding behavior and ecosystem functioning are affected by environmental stressors. However, stress responses are traditionally studied on the species level, while perturbations of functionality (e.g. animal behavior) can cascade toward ecosystem processes and therefore ecologically much more relevant, but virtually unknown. Likewise, solar radiation is an important abiotic variable that specifically may affect bacterial communities (e.g. Baldy et al., 2002; Piccini et al., 2009; Zepp et al., 2011). Solar radiation, and especially UV radiation (280-320 nm) is known to have detrimental effects on DNA (e.g. Santos et al., 2012a), or change the chemical composition and palatability of organic compounds by photodegradation (e.g. Engelhaupt et al., 2002; Sulzberger and Durisch-Kaiser, 2009). Such changes in the chemical composition of OM may subsequently cascade toward shifts in bacterial community composition due to the interplay between bacterial resource niches (i.e. the type of substrates that are utilized) and available resources (e.g. Salles et al., 2009). However, the effects of solar radiation on bacterial communities residing in sediments remain completely unexplored.

The significance of invertebrate species composition for detritus processing is a matter of intense debate. However, widely different measures are used to identify detritus processing, suggesting a scientific dilemma. Currently, detritus processing is typically evaluated with a number of functional parameters. These include bacterial activity (Mermillod-Blondin and Rosenberg, 2006; Nogaro et al., 2009) and (functional) diversity (Bertics and Ziebs, 2009; Salles et al., 2009; Gravel et al., 2011), the geochemical characteristics of the sediment (Mermillod-Blondin et al., 2002; Solan et al., 2004; Meysman, 2006; Birchenough et al., 2012) and measures of cellulose decomposition (Boulton and Quinn, 2000; Tiegs et al., 2008; Young et al., 2008; Imberger et al., 2010). However, these functional parameters are rarely studied simultaneously, and therefore the relative importance, reliability and cohesion remain uncertain. Experiments are thus required that test the predictive potential of invertebrate functional metrics in relation to OM processing.

*Hard substrates: Mangrove roots*

A great diversity of hard substrates can be found in continental waters and seas. Bed rock and coarse mineral debris (gravel and boulders) offer a habitat for detritus feeders, but organic substrates such as wood debris in rivers and tree roots in riparian and coastal habitats are important stimulants for detritus processing, as detritus accumulation around extensive root systems may also produce organic substances in situ that can directly affect the associated fauna.

For the present study roots of the mangrove *Rhizophora mangle* were selected. Mangroves form the dominant vegetation in tidal, saline wetlands along (sub-)tropical coasts (Chapman, 1976; Tomlinson, 1986). Submerged portions of mangrove aerial roots are dominated by sponges (Sutherland, 1980; Ellison and Farnsworth, 1992). Sponges are efficient filter feeders and mangrove associated sponges primarily feed on mangrove-derived particulate matter and dissolved organic matter (Granek et al., 2009).

Mangrove-derived OM, originating from decaying leaves and leachates from mangrove roots, is composed mainly of tannins and polyphenolic compounds (Maie and Jaffe, 2006), in which concentrations of tannins and polyphenols may vary depending on tissue, growth stage, and environmental conditions (Northup et al., 1998; Lin et al., 2006). Tannins are a group of secondary metabolites that is known for their anti-microbial and anti-herbivore activity (Cameron and LaPoint, 1978; Alongi, 1987; Scalbert, 1991; Arnold and Targett, 2002, Erickson et al., 2004), and it may adversely affect associated macrofaunal abundance (Lee, 1999). Since mangrove sponges mainly feed on mangrove-derived organic matter (Ellison and Farnsworth, 1996; Granek et al., 2009), it is possible that mangrove-derived DOM influence sponge physiology or larval settlement, and subsequently alters species distributions.

The composition of mangrove-associated sponges is relatively species poor, heterogeneous, and very distinct from sponge communities living on connected, nearby reefs (e.g. Van Soest, 1978, 1980, 1984; Wulff, 2004). The mechanisms that underlie this distinction remain uncertain to date (for review see: Wulff, 2012). Transplantation of typical reef species to mangrove roots results in the rapid deterioration of the transplanted sponges (Farnsworth and Ellison, 1996; Wulff, 2004; Pawlik et al., 2007). It has therefore been argued that abiotic factors (such as salinity; wave exposure; particle suspension) are of prime importance for sponge survival and perseverance in mangroves ecosystems, but it remains uncertain which abiotic factor is the key controlling variable (Pawlik et al., 2007; Wulff, 2012).

Sponges form close associations with symbiotic microorganisms. Various molecular studies have demonstrated that sponges host a diverse, and largely host specific symbiontic community (e.g. Taylor et al., 2007, and references therein), although the ecological and evolutionary nature of these communities remains uncertain to date (Thacker and Freeman, 2012). Evidence is now accumulating that sponge-hosts obtain carbon and other nutrients from their microbial associates (De Goeij et al. 2008a,b; Freeman and Thacker, 2011; Ribes et al. 2012), although the identity of key compounds and bacterial metabolic pathways remain completely unresolved (Thacker and Freeman, 2012). Increasing evidence also suggests that only a limited number of bacterial and fungal species are able to degrade complex polyphenols and tannins (Bhat et al., 1998, and references therein). It is thus possible that an interaction between recalcitrant compounds derived from mangroves and the presence of bacterial symbionts capable of degrading mangrove-derived DOM plays a pivotal role in the observed differences in species composition between mangrove and reef sponge communities. Responses of sponge-bacterial consortia to the mangrove root substrate and mangrove-derived (D)OM thus requires further evaluation.

**Aim and objectives**

Studying simultaneously a soft bottom sediment system and a hard substrate system provides the unique opportunity to study different underexposed aspects of the interaction between the (functional) composition of invertebrate and bacterial communities, and organic matter processing. Would it be possible to identify general drivers of detritus processing among distinct benthic ecosystems? The aim of this thesis was therefore to unravel interactions between the (functional) composition of invertebrate and bacterial communities, organic matter processing and abiotic variables in two contrasting benthic detrital food webs: one on soft bottom sediments and one on solid substrate ecosystems. To this purpose, the following objectives have been set:

- To evaluate the impact of OM composition on invertebrate-substrate interactions and organic matter processing

- To assess the impact of abiotic stressors on invertebrate-substrate interactions and organic matter processing
- To quantify the effect of functional diversity of bacteria and invertebrates on organic matter processing

**Thesis outline**

To meet the objectives of the present study, two series of experiments were performed on organic matter processing in two contrasting benthic habitats.

*Part 1 – Invertebrate-substrate interactions in soft bottom sediments*

The first set of experiments focused on invertebrate-substrate interactions in soft bottom sediments. Evaluating the importance of invertebrate functional diversity, especially their bioturbation behavior, for bacterial communities and detritus processing, requires manipulation of the invertebrate community, and therefore these experiments were performed in laboratory microcosms and outdoor mesocosms.

Bioturbation/feeding activities of invertebrates in sediments are known to influence decomposition rates. However, direct effects of invertebrates on bacterial communities and detritus processing remain ill-defined, mainly because identifying interactions between invertebrates and sediments is methodologically challenging. Chapter 2 therefore evaluated whether bioturbation/feeding strategies of aquatic invertebrate species differentially affects detritus processing and benthic microbial community structure and tested the utility of redox potential (Eh) profiles as biogeochemical signatures of the types of bioturbator species in laboratory microcosms.

Since chemical stressors may decouple links between biodiversity and ecosystem processes, Chapter 3 evaluated how toxicants affect the functional links between invertebrate bioturbation and ecosystem functioning. To this purpose, the effects of the model toxicant copper on two functionally distinct macrofauna species (*Asellus aquaticus* and *Tubifex* spp.), detritus processing and microbial activity and metabolic diversity were determined in 5–day microcosm experiments. The effect of altered locomotion and activity and reduced bioturbation were assessed with spatio-temporal redox (Eh) profiles in the upper sediment layer.

Those who study biodiversity effects on OM processing would benefit from standardized ways of measuring detritus processing. One standardized proxy with a chemical composition that can be easily adjusted for experimental purposes is required. Therefore, in Chapter 4 the performance of a novel decomposition and consumption tablet (DECOTAB) consisting of cellulose powder embedded in an agar matrix to evaluate microbial decomposition and invertebrate feeding was tested.

This chapter describes the preparation of the newly developed DECOTABs and evaluates some potential applications in laboratory microcosms and outdoor mesocosms.

Potential effects of solar radiation on bacterial communities residing in sediments remain completely unexplored. Chapter 5 investigated the effect of solar radiation on the functional composition of bacterial communities in shallow aquatic sediments, and compared the effect of light and dark incubation on bacterial metabolic diversity in sediment microcosms containing two different substrates: recalcitrant peat and palatably fresh plant biomass.

Several parameters are at hand to quantify the decomposition process, but these are seldomly studied in coherence and mainly rely on laboratory experiments. Chapter 6 tested therefore whether several functional parameters measured in multispecies invertebrate assemblages in outdoor mesocosms could be predicted from their responses to single invertebrate species experiments under laboratory conditions. To this purpose, bacterial functional diversity and activity, sediment redox potential and DECOTAB mass loss were measured in laboratory microcosms and outdoor mesocosms in the presence of single invertebrate species and manipulated multi-species assemblages.

*Part 2 – Sponge-environment interactions in mangrove stands*

The second set of experiments focused on the role of root substrate as driver of sponge community composition in mangrove ecosystems. Experiments were performed in a tropical mangrove ecosystem and an adjacent reef.

Chapter 7 aimed to quantify the diversity of mangrove associated sponges in the inner bays of Curaçao and Aruba, and explored correlations between a set of physico-chemical variables and sponge distributions. Tannin concentrations of selected mangrove roots were compared to sponge cover and tested as a possible driver of local heterogeneity. A positive relationship between sponge coverage and tannin concentrations in roots was observed, but the reason for this observation remained uncertain. The objective of Chapter 8 was therefore to evaluate whether tannins play a role in sponge recruitment and whether mangrove roots enhance production of tannins and total phenolics in response to sponge colonization. These aspects were addressed by performing in situ recruitment and translocation experiments.

Chapter 9 tested the hypothesis that tannin-degrading microorganisms within the endobiontic community of mangrove sponges may be partly responsible for the structural differences in reef and mangrove sponge communities. To test this assumption, the presence of tannin-degrading organisms in a random set of species collected from mangrove roots and a nearby reef by assaying tannase activity and evaluated whether endobionts were able to grow on artificial substrate containing mangrove root extracts was explored qualitatively.

To discriminate the role of habitat, substrate and symbiotic bacteria in driving sponge distributions, Chapter 10 monitored survival and condition of typical mangrove and reef sponge species after in situ reciprocal transplantation to DOM-releasing mangrove roots and DOM-free surrogate roots (PVC tubes) in both mangrove and reef environments. Next, the structure and stability of the symbiotic bacterial community in the sponge host before and after transplantation were determined. Finally, the carbon utilization patterns of the symbiontic bacterial communities of the individual sponge species were analyzed.

Chapter 11 tested whether mangrove-DOM leachates from roots are responsible for the observed deterioration of reef species transplanted to mangrove roots. To this end, a typical reef species and a typical mangrove species were transplanted to mimicry substrates containing mangrove root extract and to control substrates without extract. Mangrove DOM was also injected directly into tissues of both sponge species.

Finally, Chapter 12 evaluated the interaction between biotic and abiotic components of two contrasting benthic detrital food webs and attempted to identify general drivers of OM processing in benthic detrital food webs.