Marine Macrophytes as Foundation Species

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Preface

Marine macrophytes (macroalgae, seagrasses, and mangroves) comprise thousands of species distributed in shallow water areas along the world's coastlines. They play a key role in marine ecosystems regarding biodiversity and energy flow. A large proportion of macrophyte species can be characterised as ecosystem engineers—organisms that directly or indirectly affect the availability of resources to other species by modifying, maintaining, and creating habitats.

This book is divided into three main themes:

- *Marine macroalgae and seagrasses as sources of biodiversity* gives an overview of the diversity of the main organisms associated with macrophytes, and their functional role and interactions within their hosts.
- *Primary and secondary production of Macrophytes* synthesizes research on food web structures derived from/or associated with, macrophytes and the transfer of macrophytic primary and secondary production from one ecosystem to another.
- *Threats to macrophytic ecosystem engineers* addresses human-induced effects including eutrophication, physical destruction, invasive species, and global warming.

The main features of the book are: Discusses how microalgae and seaweeds contribute to biodiversity; synthesizes research findings on macrophytes as a source of food; covers threats to marine ecosystems caused by human activities; serves as the first book to cover the value of macrophytes for the well-being of marine habitats.

The book is among the first one to concentrate on the value of macrophytes for the well-being of marine habitats. The book is aimed at academics but may also be useful for students, policy makers, and laymen alike.

I dedicate this book to Agnar Ingólfsson and Ragnar Elmgren; both had a strong impact on my career in marine biology.

Emil Ólafsson
Palma de Mallorca, Spain
July 6th, 2016

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I. Macroalgae and Seagrasses as a Source of Biodiversity

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Microbial Biodiversity Associated with Marine Macroalgae and Seagrasses

Franz Goecke and *Johannes F. Imhoff* [a]

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Introduction

Macrophytes are the main primary producers of coastal ecosystems, which include many habitat-forming species with vital ecological importance. Macroalgae and seagrasses as any other macroorganisms in this ecosystem are in permanent contact with microbes. Seawater contains millions of microorganisms per ml of bacteria, cyanobacteria, fungi, microalgae and protists (Goecke et al. 2010), which eventually interact with every single species of macrophytes during the entire lifetime till the next generation. In the marine environment microbes are found in the immediate surroundings, floating in the water or along with currents and tides. The aquatic environment favors the formation of biofilms on surfaces. Besides every mechanical, chemical and physical defense, microorganisms can be found on every living surface in the aquatic environment, and this includes marine macrophytes as well (Wahl et al. 2012).

Such epibiotic biofilms have a huge potential to affect the biology, ecology and fitness of their host (the basibiont). Many direct and indirect effects of epibiotic biofilms have been described, many more can be expected to exist, but the consensus of most investigations is that the presence of biofilms alters the substratum physically and chemically, and that they have the capacity to modulate (reduce, enhance,

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select) the recruitment of other microbes and macroorganisms (recently reviewed by Wahl et al. 2012).

Macrophytes are especially susceptible to epibiosis and are typically covered by diverse microbial communities that normally include bacteria, microalgae, fungi and diverse protists (Sieburth and Thomas 1973, Raghukumar and Raghukumar 1992, Kohlmeyer and Volkmann-Kohlmeyer 2003, Neuhauser et al. 2011). Uncontrolled biofilm formation produces a permanent threat to macrophytes (see Goecke et al. 2010), although the consequences of epibiotism and endobiosis for the host macrophyte are not always necessarily negative, but instead depend on the community context. Hence, such intimate associations can range from mutualism through commensalism and parasitism in an almost continuous spectrum (Potin 2012).

The colonization of marine macrophytes is quite variable and dense microbial populations (up to 10^6 cells per cm^2) may be found. Density and composition of epibiotic biofilms vary at different scales: Among host species, conspecific host individuals, body regions of a host individual, among habitats, seasons and during the life cycle (Wahl et al. 2012). Microbes and basibionts are not passive players in the colonization process. The macrophyte basibiont can exhibit a variety of defenses against microbial fouling and epibionts. This resistance can be generally mediated by either structural or chemical means, with both mechanisms interfering with the attachment, colonization (and penetration) and dispersion of microorganisms, spores or propagules (Potin 2012). A wide variety of macroalgae and seagrasses have shown to produce diverse antibiotic (antifungal, antibacterial, antiprotozoal secondary metabolites) and antifouling compounds, which may allow the host to modify the composition of the associated microbial populations (Lam et al. 2008, Olson and Kellogg 2010, Goecke et al. 2012a, Grosser et al. 2012). The epibiotic microorganisms can produce biologically active metabolites as well or modify those available and thereby enhance the antifouling protections as suggested by different authors (Armstrong et al. 2001, Goecke et al. 2013a).

Besides some detrimental effects, a wide range of beneficial and neutral interactions have been observed between macrophytes and microorganisms. Many of the microorganisms are specifically associated with their hosts and may enable them to expand physiological capacities (Hollants et al. 2013a), to survive adverse environmental conditions and to overcome competitors.

After different evolutionary and ecological processes, those negative and positive interactions, together with the great diversity of microorganisms and macrophytes may be responsible for differences on microbial associations with different co-occurring hosts (Goecke et al. 2012a, 2013a,b). Macrophytes sustain therefore a huge biodiversity of microbes that need to be explored. In this chapter, we focus on recent advances in this field, especially on interactions between bacterial and fungal communities with macrophytes, and point out some urgent questions for future research. These microbial groups were chosen because they are the most abundant in this habitat and their relation to macrophytes has been studied more intensively than that of other microbes. In addition, many other 'minor' groups of microorganisms which interact with macrophytes, e.g., oomycetes, labyrinthulids, flagellates, heliozoans, ciliates, amoebozoans, thraustochytrids, foraminiferans, and phytomyxids were not considered because of limited space. However, the prokaryotic domain of the Archaea is included.

During the last decades, a diversification and intensification of aquaculture, the sustained nutrient enrichment from land-based activities, pollution, biological invasions, ocean acidification, global warming and climate change, among others, have demonstrated to strongly affect biotic interactions (Eggert et al. 2010, Gachon et al. 2010, Case et al. 2011, Wernberg et al. 2011, Williams 2007, Potin 2012). This points to the necessity to develop an understanding on the microbial communities specifically associated with macrophytes. Which are the ecological processes that are involved? And how can these microbial communities affect and be affected by the habitat-forming and principal primary producers of the coastal ecosystems.

Marine bacteria

Studies of bacteria associated with macrophytes were reported since late 19th century (Hollants et al. 2013a). During the last five years an interesting number of publications have dealt especially with bacteria-macroalgae relationships, trophic and chemical interactions and the associated microbial communities, which are discussed in this chapter. The relationship between macrophyte and bacteria in which the host provides nutrients and habitat, while the bacterial community promotes macrophyte growth and protects the host against pathogens, has been elaborated over the last 20 years (Hollants et al. 2013a). While several bacterial species have been identified as causative agents of diseases of macroalgae, many bacterial associations are clearly beneficial for the algal host (Kurtz et al. 2003, Matsuo et al. 2003, Amin et al. 2009, Goecke et al. 2010, Seyedsayamdost et al. 2011, Grant et al. 2014). Recent comprehensive reviews have examined the body of literature available on bacteria associated with macroalgae and seagrasses, which is largely built on cultivation-based studies (see Duarte et al. 2005, Goecke et al. 2010, Egan et al. 2013, Hollants et al. 2013a). On one hand, these suffer from the known limitations of adequately describing the composition of natural microbial communities by cultivation-dependent approaches (Friedrich 2012, Fig. 1), on the other hand, they offer plenty of possibilities to test ecological hypotheses in controlled experimental setups such as reinfection experiments with defined pathogens.

Biofilms on the surface of marine organisms are usually dominated by prokaryotes (Bacteria). Bacterial biofilm with cell densities of 10^2 to 10^8 cells cm^{-2} have been found on different macroalgae (see Dobretsov et al. 2006, Bengtsson et al. 2010, Wahl et al. 2010); similar values have been determined for roots and leafs of different seagrass species (Kurilenko et al. 2001, García-Martínez et al. 2005). Macrophyte surfaces as a microbial habitat display several advantages and have characteristic properties that influence their association with microbes such as nutrient supply, defense against competitors, protection from damaging ultraviolet radiation and predation, among others (Goecke et al. 2013a); therefore they are highly attractive and competitive marine environments.

The analysis of microbial communities associated with macrophytes is still in its infancy and in particular functional interactions are not known. The molecular microbial ecology of macroalgal or seagrass hosts is a rather young field with only small datasets available but with large potentials to influence theory building in microbial ecology and host–microbe interactions (Friedrich 2012). Nevertheless, in most cases molecular investigations have confirmed the outcome of initial cultivation
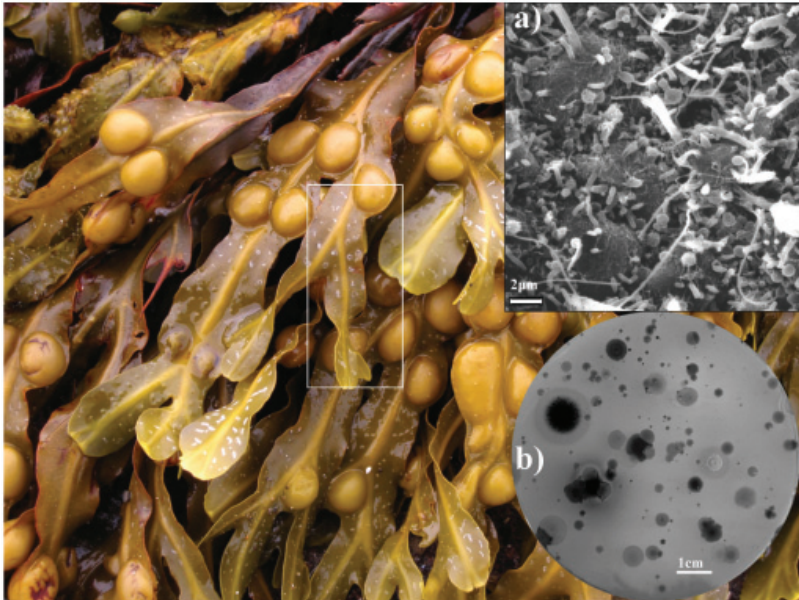
Figure 1. Biofilm on the common brown macroalgae *Fucus vesiculosus* in the Baltic Sea. (a) Scanning electron microphotograph (SEM) with details of the microbial biofilm over the surface of the thalli of the alga. (b) Petri dish showing the growth of different colonies of microorganisms after processing a small piece of the alga (indicated by the white box). Clearly just a selection of microorganisms is growing on a particular nutrient medium, which does not reflect the true diversity and real abundances associated with the macroalgae.

studies, i.e., that the attraction of bacteria by macrophytes turns out to be highly specific (Hollants et al. 2013a). While the composition of the bacterial flora can change over seasons, life span and different thallus-parts as a result of biotic and abiotic factors (Crump and Koch 2008, Staufenberger et al. 2008, Bengtsson et al. 2010, Tujula et al. 2010), marine macrophyte generally associate with specific bacterial communities that differ significantly from those occurring in the surrounding seawater or on inanimate (and undefended) substrata in close vicinity (Dobretsov et al. 2006, James et al. 2006, Longford et al. 2007, Lachnit et al. 2009, Bengtsson et al. 2010, Burke et al. 2011b, Hollants et al. 2013a).

Recent research also confirms that different species of marine macrophyte in the same habitat support differently composed bacterial communities (Lachnit et al. 2009, 2011, Trias et al. 2012a), while specimens of the same macrophyte even in different environments tend to be associated with highly similar bacterial communities (Uku et al. 2007, Staufenberger et al. 2008, Lachnit et al. 2009). Even though a consistent bacterial core community at higher taxonomic levels (i.e., of Proteobacteria and Bacteroidetes, Table 1) was observed on different macroalgae (see Staufenberger et al. 2008, Tujula et al. 2010, Burke et al. 2011b), phylogenetically closely related macroalgae may not necessarily harbor the same bacterial taxa (Hollants et al. 2013a). Certain bacterial symbionts are sensitive markers potentially useful to distinguish genetically similar macroalgae as suggested by Balakirev et al. (2012).

Table 1. Bacterial phyla associated with macrophytes as determined by culture-independent methods. A consistent bacterial core community formed by Proteobacteria and Bacteroidetes was observed on different macrophyte samples (based on Cifuentes et al. 2000, Weidner et al. 2000, Jensen et al. 2007, Uku et al. 2007, Crump and Koch 2008, García-Martínez et al. 2009, Balakirev et al. 2012, Bengtsson et al. 2012, de Oliveira et al. 2012, Garcias-Bonet et al. 2012, Fernandes et al. 2012, Hollants et al. 2013a,b, Wahl et al. 2012, Aires et al. 2013, Miranda et al. 2013, Sweet et al. 2013, Wang et al. 2013, Stratil et al. 2014).

	Dominant	Common	Rare
Macroalgae			
	Alphaproteobacteria	Firmicutes	Chloroflexi
	Gammaproteobacteria	Actinobacteria	Chlorobi
	Bacteroidetes	Betaproteobacteria	Lentisphaerae
		Deltaproteobacteria	Epsilonproteobacteria
		Planctomycetes	Deinococcus-Thermus
		Cyanobacteria	Spirochaetes
			Verrucomicrobia
			Fusobacteria
			Tenericutes
			Acidobacteria
Seagrasses			
	Gammaproteobacteria	Planctomycetes	Chloroflexi
	Alphaproteobacteria	Betaproteobacteria	Holophaga-Acidobacterium
		Bacteroidetes	Spirochaetes
		Epsilonproteobacteria	Actinobacteria
		Verrucomicrobia	Cyanobacteria
		Firmicutes	Deferribacter
		Deltaproteobacteria	Nitrospira

Studies of seagrasses have particularly been concerned with specific functional groups of bacteria (Table 1), such as sulfate-reducing bacteria (Küsel et al. 1999, Nielsen et al. 1999, Finster et al. 2001), nitrogen-fixing bacteria (Kirshtein et al. 1991, Hansen et al. 2000, Bagwell et al. 2002) and acetogenic bacteria (Küsel et al. 1999), whereas only in a few studies on the general bacterial diversity has been considered by using molecular analyses (see Cifuentes et al. 2000, Weidner et al. 2000, Jensen et al. 2007, Uku et al. 2007, Crump and Koch 2008, Garcias-Bonet et al. 2012). The large similarities observed at higher taxonomic ranks among the associated-microbial communities of macroalgae and seagrasses decrease notoriously at lower ranks of bacteria (for details see Table 1 and references therein) and point out more specific associations at the lower ranks.

In terms of biodiversity and because of this specificity, macrophytes are an interesting environment for discovery of new bacterial taxa. In the case of algae, several new bacterial species (101 spp.) and new genera (36 genera) have been isolated (reviewed by Goecke et al. 2013b). Furthermore, the authors conducted a phylogenetic study (supported by phenotypic information) of those new species based on 16S rRNA gene sequences and obtained a similar phylogenetic distribution as found in cultivation-independent molecular studies (as in Table 1). Also seagrasses were a source of a number of new bacteria, e.g., *Desulfomusa hansenii* (Finster et al. 2001),

Desulfovibrio zosterae (Nielsen et al. 1999), *Granulosicoccus coccoides* (Kurilenko et al. 2010), and diverse *Marinomonas* species (Lucas-Elío et al. 2011).

It feels like home: The phyto(phyco)-sphere

As mentioned before, the establishment of epibiosis is not a simple process, and various physical and chemical properties of the host surface, as well as interactions among the settlers are determinants of the formation of specific communities (e.g., García-Martínez et al. 2005, Wahl et al. 2010, 2012). The ecological function of different macrophytes can be similar as they are primary producers that provide physical habitat and trophic support to other species which in turn are important food items for other organisms (Thomsen 2010). However, species-specific attributes with respect to the phenology, morphology and biology of each taxon can influence the structure of associated communities differently (Drouin et al. 2011).

The phytosphere is a unique and attractive environment, which consists of the phyllosphere, the endosphere and the rhizosphere (the last one normally absent in macroalgae with exception of, for example, some Bryopsidales). Each of these three habitats provides a considerably diverse physical, chemical and biological environment, and as a consequence can support a wide range of different microbial groups (Saito et al. 2007).

Thallus morphology and microtopography of the macrophyte surface play an important initial role in the colonization and association with the microbiota and invertebrates (Wahl et al. 2012) as does the surface chemistry of a macrophyte, which is the sum of exuded secondary metabolites and extracellular exopolymeric substances present on the thallus (Grosser et al. 2012). This complex mixture of compounds, presumably in equilibrium with the ambient water body, is a primary source of nutrients for those bacteria attached to the surface (Lachnit et al. 2010, Salaün et al. 2012). Rhizoplane bacteria obtained from the roots of *Zostera marina* demonstrated chemotaxis towards root exudates, in particular amino acids (Wood and Hayasaka 1981). Bacteria may use the host not only as a surface for settlement but also as a source of substrates for their own nutrition. The utilization of substrates produced or released by macrophytes, including (structural) polysaccharide components of cell walls, e.g., alginate, agar, carrageenan, cellulose, fucoidan, laminaran, porphyran, ulvan, is an important aspect of surface colonization by bacteria (Bengtsson et al. 2011, Salaün et al. 2012). Such macromolecular composition is characteristically different among the major evolutionary lineages, linking specific life style or nutritional habits to specifically encountered biopolymers (Goecke et al. 2013b). Hence, each algal/seagrass species can be considered to be a unique micro-environment.

It is important to consider that macrophyte structural complexity is an important factor influencing the abundance and taxon richness of microfauna, e.g., rotifers, copepods, cladocerans (Lucena-Moya and Duggan 2011), macrofauna, e.g., amphipods, mollusks, annelids and even fishes (Tuya et al. 2009). Macrophytes with a more complex morphology such as those presenting diverse ramifications as *Ceramium*, *Cystoseira* and *Corallina* spp., support a greater diversity and abundance of associated macroorganisms in comparison to macrophytes with flat and simple fronds such as

Chondrus, *Porphyra* and *Palmaria* spp. (Chemello and Milazzo 2002). The reasons suggested are a reduction of mortality by predation, reduction of physical stress (wave action, desiccation, UV irradiation), by accumulating species transported passively by currents; and by proportioning a major surface for perifiton and other small food items (Dean and Connell 1987, Warfe and Barmuta 2004). Surprisingly, its effect on microbial communities remains uninvestigated. The accumulation of potential predators (microfauna) could negatively affect the abundance of microorganisms, but also the facilitation of settlement, accumulation of microorganisms and organic matter, along with the presence of a variety of microhabitats could instead promote diversity and abundance of microbes.

One hand clean the other: Bacterial interrelationships in the seagrass rhizosphere

Bacterial abundance is generally higher in sediments from seagrass meadows than in adjacent unvegetated sediments (Duarte et al. 2005, and references therein). Seagrasses develop a complex rhizosphere, typically deploying several hundred meters of root material per square meter of seagrass meadow and occupying up to 80% of the sediment space (García-Martínez et al. 2005). These sediments contain active bacterial communities that consume molecules excreted by plant roots such as organic molecules (amino acids, sugars and organic acids) and gases (O_2, N_2 and CO_2). In return, microbial processes, including organic matter mineralization, phosphorous solubilization and nitrogen fixation, provide nutrients for the macrophytes (Hansen et al. 2000, Crump and Koch 2008).

Inside the intricate seagrass meadow, the increase in physical structure complexity affect sedimentation, turbulence and water flow, trapping sediments and stimulating the accumulation of organic matter below the meadows (Fig. 2). This organic matter is available for microbial processes and can easily develop anoxic conditions. As a reflection of this, sulfate-reducing bacteria, the predominant bacteria involved in anaerobic degradation of organic matter, proliferate and consequently sulfate-reduction is increased with the posterior accumulation of toxic sulfide (Duarte et al. 2005, Jensen et al. 2007, and references therein). Anoxic and toxic conditions can be detrimental for seagrass growth and survival. However, these plants release oxygen through the roots and thereby (partially) prevent anoxic conditions. This oxygen may be crucial for host-associated microbial processes and for aerobic bacterial metabolism (Duarte et al. 2005). Some microbes may have beneficial effects on seagrasses by metabolizing toxic substances in the rhizoplane, e.g., sulfide-oxidizing bacteria using nitrate as electron acceptor (Küsel et al. 2006) or ammonia-oxidizing bacteria (Ando et al. 2009). The presence of bacteria related to well-known nitrogen fixers is especially interesting because these may have a beneficial role in the establishment, growth and survival of macrophytes (such as *Posidonia oceanica*) in oligotrophic environments (Kirshtein et al. 1991, Bagwell et al. 2002, Garcias-Bonet et al. 2012); with a resulting mosaic of microhabitats and diverse coexistent phytosphere-microbial communities.

The effects of seagrasses on sediment organic matter and oxygen inputs modify the bulk biogeochemical redox conditions in the sediments. Plant root exudates can
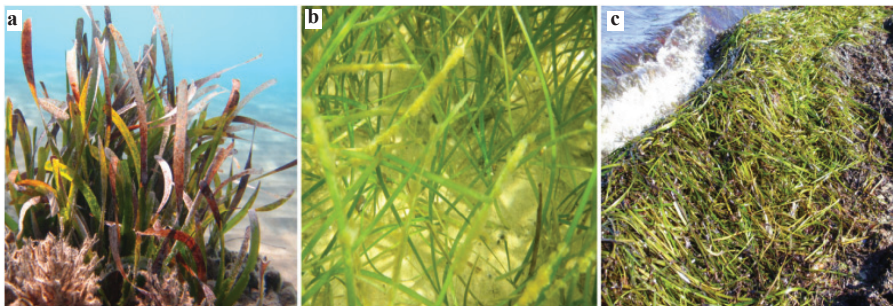
Figure 2. Habitat-forming seagrasses in the marine environment. (a) A patch of *Posidonia oceanica* growing in the Mediterranean Sea. Sometimes these macrophytes can form 'island' between clear sandbanks which provide shelter and refuge for diverse macro- and microorganisms. (b) Detail into a seagrass meadow of *Zostera marina* in Germany, Baltic Sea. The intricate meadow increases in physical structure complexity, trap sediments and stimulate the accumulation of organic matter below the meadows, reduce detection of preys by bigger predators and proportionate a major surface for perifiton. (c) Once dislodged (e.g., by storms, herbivores or death) piles of the seagrasses may accumulate on the beach and form very special conditions in support of a large number of macro- and microorganisms.

selectively stimulate and inhibit microbial growth and thus foster the establishment of specific microbial communities (Jensen et al. 2007). Bacterial rhizosphere communities can vary in composition according to plant species, growth stage, root exudates, and available carbon source (Kurtz et al. 2003, and references therein). Nevertheless, the role of bacteria (as in sulfur cycling) can also determine the health and therefore the growth rates of marine angiosperms (Garcias-Bonet et al. 2012). Recent declines in seagrass distribution underscore the importance of understanding microbial community structure-function relationships in the seagrass rhizosphere that might affect the viability of these macrophytes (Küsel et al. 1999), and may be vital for restoration programs. Moreover, van der Heide et al. (2012) hypothesized that seagrass meadows may provide an optimal habitat for certain bivalves (Lucinidae, Bivalvia) and their bacterial symbionts by indirectly stimulating sulfide production by increasing organic matter content and by providing oxygen through radial oxygen release from the roots. In turn, those lucinids remove sulfide, which could relieve any stress caused to seagrass growth by sulfide. In this way a three-stage symbiosis is formed supporting the success of seagrass meadows.

A new perspective: Specific function instead of species-specific

In order to differentiate between community structure and function in the bacterial assemblages associated with the green alga *Ulva australis*, a new perspective came from Burke et al. (2011a), who analyzed metagenomic sequences. Despite high phylogenetic variability in the microbial species composition associated with that alga (see Tujula et al. 2010), the authors discovered little functional variability in the functional composition (measured as presence of functional gene clusters). Phylogenetically different bacterial species (or strains)—able to colonize one particular host species—that can carry out similar metabolic and other functions apparently

compete with each other in the colonization of particular algal surfaces (Burke et al. 2011a). This suggests that the assembly, structure and ecological role of bacterial communities in the future should be described preferentially at the functional level. In this sense, different bacterial species and strains that carry out similar metabolic functions were found to colonize similar algal taxa or algal groups (Goecke et al. 2013b). Also, studies on different seagrass species revealed that a variety of microorganisms able to fix di-nitrogen are associated with the phyllosphere and rhizosphere of seagrasses (Kirshtein et al. 1991, Cifuentes et al. 2000, Weidner et al. 2000, Uku et al. 2007). Since for the host (and its interactions with the environment) microbial function matters more than phylogenetic composition, investigations at the functional level based on genomic or metabolomic information should become more prominent in the future (Wahl et al. 2012).

Traditionally, classification of bacteria has relied on cultivation. Since only a selected fraction of bacteria will grow on a specific medium, the cultivated fraction of bacteria may not reflect the abundant/diverse bacterial populations of their habitat (Friedrich 2012, Fig. 1). Metagenomic approaches on the other hand can reveal the complex biodiversity of the bacterial assemblages, but give information on the functional aspect only to a rather limited extent. There is no doubt that we are only starting to discover the specific and complex relationships of macrophytes and bacteria in nature. Interactions between bacteria and algae (macrophytes) are thought to be important in controlling the dynamics of both communities and yet are barely understood at the species and functional level (Grossart et al. 2005). Moreover, it is likely that macrophyte-bacterial relationships will change as the host ages (Seyedsayamdost et al. 2011). Recent technical advances in environmental microbiology have enabled the evaluation of microbial diversity and/or genomic diversity using rapid, simple and less biased culture-independent molecular techniques (Saito et al. 2007). These changes include typically commensal epiphytic microorganisms that are common on the surface but could become detrimental if entering into the macrophyte tissue (Goecke et al. 2010) or if the host/epibiotic microbial community are strongly affected by environmental factors such as ocean acidification or warming (Eggert et al. 2010, Campbell et al. 2011, Case et al. 2011, Fernandes et al. 2012). A better insight into these mutualistic and pathogenic interactions is necessary for understanding and predicting algal bloom formations, disease outbreaks, and the response of populations of macrophytes and bacteria to changes in their environment (Amin et al. 2009).

Marine archaea

The dogma until around 1992 was that Archaea inhabit mainly 'extreme' environments, inhospitable to most other forms, but later, based on cultivation-independent studies, they were surprisingly found to be abundant in coastal and open-ocean waters (DeLong 2007). Archaea were detected in association with different marine organisms, sponges and corals (Olson and Kellogg 2010) and also with the rhizosphere of different macrophytes, e.g., *Ruppia maritima* (Trias et al. 2012b).

With respect to seagrasses, Cifuentes et al. (2000) identified members of Euryarchaeota and Crenarchaeota in sediments taken from a *Zostera noltii* meadow,

and later the authors were able to grow (although not in pure culture) one marine non-extremophile Crenarchaeota (Cifuentes et al. 2003). In a recent study, seasonal changes in abundance of ammonia-oxidizing archaea were observed with quantitative PCR within sand of a *Zostera marina* meadow (Ando et al. 2009). The biogeochemical implications can be essential: Nitrification is important to sustain and control the nitrogen cycle in eelgrass zones and ammonium can be toxic to the seagrass species. Archaea have been detected on macroalgae only recently, in mesophotic depth in the Mediterranean Sea (Trias et al. 2012a). But this may be only as a result of lack of research in this field. Only few investigations have been made on macrophyte-associated archaea and information on community compositions (abundance and diversity) of archaea is lacking. Besides the ammonia-oxidation, no other potential beneficial or detrimental effects of archaea for the macrophytes have been postulated. One of the likely reasons for this is the fact that most microbial ecology studies of macrophyte surfaces have been culture-based and proper culture conditions for these archaea are lacking (Olson and Kellogg 2010). It is evident, however, that archaea other than methanogens may be an important part of the prokaryotic community in the marine phytosphere (Cifuentes et al. 2000), though there is a massive gap of knowledge on archaea-macrophyte interactions.

Marine fungi: Filamentous fungi and yeasts

The occurrence of fungi in the marine environment was first reported late in the 19th century, therefore marine mycology is considered as a recent science field (Shearer et al. 2007). Fungal species in the marine environment include those that are adapted to complete their life cycles in marine aquatic habitats and are not found outside of the aquatic environment <'residents', 'obligate marine'>, those that occur in water fortuitously by being washed or blown in <'transients'>, and those 'facultative' marine species, which may grow in marine as well as in freshwater or terrestrial habitats (Kohlmeyer and Kohlmeyer 1979, Shearer et al. 2007).

Fungi from coastal and marine ecosystems are often neglected. Although marine fungi grow on most organic substrates occurring in oceans and estuaries, the fraction of cultivable isolates is very low (1% or less) with regard to the overall estimated biodiversity (Hawksworth and Rossman 1997, Rateb and Ebel 2011). Nevertheless, fungi have key roles in the marine environment and act as decomposers in nutrient turnover of organic matter (Sridhar et al. 2012). They are of particular interest due to their ecological significance and interactions (Apt 1988, Zuccaro et al. 2003). Pathogens, parasites, saprobes, and mycobionts are the predominant fungi of marine macrophyte communities. However, there is little data available about the ecology of these organisms (Zuccaro et al. 2008). It is often difficult to define the ecological role of associated-fungi in coastal habitats, especially because of unstable relationships (symbiotic, to parasitic and saprophytic) over time (Kohlmeyer and Kohlmeyer 1979).

It has been suggested that macroalgae harbor the highest diversity of aquatic fungi, closely followed by sponges and mangroves as habitats (Rateb and Ebel 2011). Reviews of the literature on algicolous fungi have been published by Andrews (1976), Kohlmeyer and coworkers (1973, 1979, 1981, 1991 and 2003) and Zuccaro and Mitchell (2006).

Of particular interest are permanent symbiotic associations between systemic marine fungi and macroalgae in which the habit of the alga dominates <mycophycobioses>, because most probably they are considered as primitive lichenizations (Kohlmeyer 1973). In this sense, the interactions between the brown macroalgae *Ascophyllum nodosum* and *Pelvetia canaliculata* and their obligate symbiont *Mycophycias ascophylli* (Ascomycetes) are of importance (Deckert and Garbary 2005, Xu et al. 2008). Their studies suggest that *M. ascophylli* may have a protecting role by increasing the growth of algal zygotes and protecting them from desiccation. Although *A. nodosum* and *M. ascophylli* have each been cultured in isolation, both grow very slowly without their symbiont and neither *A. nodosum* nor *P. canaliculata* is found in nature without the fungus (Toxopeus et al. 2011). The ecological implications of the faster growth rate are important in that this might provide an escape mechanism from herbivory (Deckert and Garbary 2005).

In comparison with bacteria, cultivation-independent studies on the diversity of fungal species associated with macrophytes are limited to only a couple of investigations (see Zuccaro et al. 2003, 2008), which do not allow comparisons or general conclusions. Most information on the fungal community 'diversity' on macrophytes is obtained from cultivation-dependent studies (Meyers et al. 1965, Miller and Whitney 1981, Newell 1981, Phillips 1982, Cuomo et al. 1985, 1988, Sathe and Raghukumar 1991, Almaraz et al. 1994, Genilloud et al. 1994, Wilson 1998, Alva et al. 2002, Devarajan et al. 2002, Loque et al. 2010, Panno et al. 2010, Sakayaroj et al. 2010, Sridhar et al. 2012, Suryanarayanam et al. 2010, Godinho et al. 2013, Mata and Cebrián 2013, Shoemaker and Wyllie-Echeverria 2013), which have gained valuable knowledge in this field, but suffered from the mentioned biases.

The most commonly encountered fungi belongs to the Ascomycota, in agreement with cultivation-based studies. Basidiomycetes, Chytridiomycota and Zygomycota are also present normally on algal samples (Eggert et al. 2010, Gachon et al. 2010, Gleason et al. 2011), but they have not usually been detected in these molecular studies. Phylogenetic analysis of *Fucus serratus* of the Atlantic coast of Germany, revealed the presence of four main ascomycete orders: The Halosphaeriales, Hypocreales, Lulworthiales and the Pleosporales (Zuccaro et al. 2003), predominantly the *Lindra, Lulworthia, Engyodontium, Sigmoidea/Corollospora* complex and *Emericellopsis/ Acremonium* types (Zuccaro et al. 2008).

While an increasing number of studies have identified fungi as important agents in the pathology of marine plants, very little is known about the mechanism of pathogenesis. Studies of these fungi have been undertaken mostly from a taxonomic approach, but they provide only few data on the host-parasite interaction, pathogenicity, predisposition and epidemiology (Andrews 1976, Kohlmeyer and Kohlmeyer 1979). Also, there are only a few studies on the asymptomatic fungal endosymbionts (Schulz et al. 2002, Suryanarayanan 2012). Recognizing the enormous undiscovered diversity in fungi, especially those associated with macrophytes, is both an opportunity and a challenge (Hawksworth and Rossman 1997). While a lot of information has accumulated on the roles of fungi in structuring and maintaining terrestrial ecosystems like forests, their role in orchestrating an important part of the marine ecosystem consisting of macrophytes is not clear (Suryanarayanan 2012). Supported by the high diversity of hosts and fungi, the extended evidence on symbiotic relationships with

(terrestrial) algae (as lichens) and land plants (in mycorrhyzal symbiosis), we can expect that many close and specific associations between macrophytes and fungi still remain to be discovered.

Marine fungi as pharmacy for macrophytes

Algicolous fungi are proposed to have important ecological functions for the host that includes providing increased resistance against biotic stresses. It is known that a number of secondary metabolites play a major role in the symbiotic life forms and that natural products derived from associated microorganisms may function as a chemical defense for the host (Armstrong et al. 2001, Goecke et al. 2013a). In this sense, protective effects are thought to be mediated by fungal-derived natural products which also makes algicolous fungi a valuable resource for new bioactive compounds (Schulz et al. 2002, Miao et al. 2012). In comparison with seagrasses, those living in association with marine macroalgae are a particularly promising source of novel natural products due to the special and diverse ecological niche in which they exist (Kohlmeyer and Kohlmeyer 1979, Flewelling et al. 2013, Godinho et al. 2013). Macroalgae are a very prolific source of bioactive secondary metabolites themselves (Goecke et al. 2010), and certain microorganisms associated with them are able to metabolize these compounds (as nutrients), detoxify them or even modify them for their own purposes.

There are only a few examples in this case. In one, bromosesquiterpenes like aplysistatin, palisading A and 12-hydroxypalisadin B (isolated from the red alga *Laurencia luzonensis*) were biotransformed by the algicolous fungus *Rhinocladiella* sp. K-001, yielding two new compounds: 3,4-dihydroaplysistatin and 9,10-dehydrobromopalisadin A (Koshimura et al. 2009). Similar experiments were made with other algicolous fungal strains of *Aspergillus, Chrysosporium* and *Hypocrea* sp. (for more information see Leutou et al. 2009, Ramesh and Kalaiselvam 2011, Yun et al. 2011). Although the specific meaning of these transformations has not been ecologically tested, biotransformation can be applied to generate new active or less toxic derivatives of bioactive natural products (Leutou et al. 2009). It offers not only possible physiological and ecological benefits to the host (i.e., more powerful chemical defenses or detoxification), but also interesting biotechnological applications. Unfortunately, most of the reports on secondary metabolites have been directed toward the examination of fungal metabolites for biomedically relevant activity, largely ignoring the ecological roles of fungal secondary metabolites in the marine ecosystem (Jenkins et al. 1998). Clearly further research at the physiological, metabolic and the molecular level is necessary to obtain better insight into the chemical ecology of host-fungal relationships (König et al. 2006).

Marine yeast: Important agents on macrophyte-degradation processes

Yeasts (a polyphyletic group of fungi) are ubiquitous in their distribution and their populations mainly depend on the type and concentration of organic materials. Their environmental role is similar to many other fungi, acting as saprophytes and pathogens

of plants and animals, and they participate in a range of ecologically significant processes in the sea (e.g., decomposition of vegetal substrates, nutrient-recycling), especially in estuarine and near-shore environments (Kutty and Philip 2008).

Yeast can be commonly found in large numbers associated with marine macrophytes (1 to 10^5 yeast cells gram of the host, see Suehiro 1960, van Uden and Castelo-Branco 1963, Sieburth and Jensen 1967, Seshadri and Sieburth 1971). Many reports describe the abundant development of yeasts, especially on decomposing macroalgae (Bunt 1955, Suehiro and Tomiyazu 1962, Patel 1975, Seshadri and Sieburth 1975, and references therein). Yeasts are truly versatile agents of biodegradation and may utilize exudates of their living (and dead) hosts. For example, different species of *Candida* assimilate a variety of macrophyte-derived compounds including alginic acid, fucoidin, fucose, galactose, glucose, mannitol and phloroglucinol (Seshadri and Sieburth 1975). In this way, macrophytes can act as an important reservoir for yeasts. For example, huge heaps of kelps (like *Macrocystis pyrifera*) when deposited above the high tide line may remain landlocked for several days and undergo decomposition. Later, when eventually washed back into the sea, the yeast population can be released into the seawater (van Uten and Castelo Branco 1963).

A literature survey revealed that investigations on marine yeasts are comparatively few and that this group of marine mycota is still poorly understood (Kutty and Philip 2008). Most of the studies on yeast associated to macrophytes are concerned with isolation of strains from fresh and natural or decomposing samples (Table 2). There are numerous reports on seasonal and host variation (Suehiro 1960, Seshadri and Sieburth 1971, Patel 1975, Summerbell 1983, Wilson 1998), but there are no cultivation-independent studies on the distribution of yeast on macrophytes. Though diverse organic extracts and compounds of macrophytes exhibited anti-yeast bioactivities which could be useful as host defense (e.g., Ballesteros et al. 1992, Choi et al. 2009), no field test have been made of ecological associations. There is currently no evidence for symbiotic relationships between yeast and macroalgae. Only recently, the yeast *Metschnikowia australis* was found in high densities in the intra-vesicular fluid of the brown Antarctic macroalga *Adenocystis utricularis*. This is an interesting habitat since the yeast is protected inside the alga against the stressful environmental conditions and probably is able to utilize photosynthetic nutrients released by the macroalga (Loque et al. 2010), but the possible ecological association between *M. australis* and *A. utricularis* and it prevalence needs further investigation with molecular methods.

A comment on marine protozoa

Protists are microscopic eukaryotic microbes that are ubiquitous, diverse, and major participants in oceanic food webs and in marine biogeochemical cycles (Sherr et al. 2007). Several studies have documented the presence of heterotrophic protists (Armstrong et al. 2000), naked amoebae (Rogerson 1991), foraminiferans (Semeniuk 2001, Debanay and Payri 2010), labyrinthulids (Bergmann et al. 2011, Garcias-Bonet et al. 2011), phytomyxids (den Hartog 1989, Neuhauser et al. 2011, Goecke et al. 2012b), thraustochytrids (Phillips 1982, Raghukumar and Raghukumar 1992), and diatoms (Sieburth and Thomas 1973, Siqueiros-Beltrones and Ibarra-Obando 1987,

Table 2. Yeast genera associated with marine macrophytes in different localities around the world. The yeast genera are: *Aureobasidium* (Au), *Candida* (Ca), *Cryptococcus* (Cr), *Debaryomyces* (De), *Kluyveromyces* (Kl), *Leucosporidiurn* (Le), *Metschnikowia* (Me), *Meyerozyma* (My), *Pichia* (Pi), *Rhodotorula* (Rh), *Saccharomyces* (Sa), *Sporobolomyces* (Sp), *Torulopsis* (To), *Trichosporon* (Tr), *Yamadazyma* (Ya), and undetermined genera are represented by (un). The numbers of species are in parenthesis. Also, the states (St) of the sources are established as decomposing sample (d), fermented sample (f), fresh from the natural environment (n) and processed sample (p).

Yeast genus	Source	St	Locality	Reference
Le	Algae (5/7), seagrass (1)	n	Canada	Summerbell 1983
un	Alga (1)	n/p	Norway	Sieburth and Jensen 1967
un	Alga (1)	d	Antarctica	Bunt 1955
un(4)	Seagrass (3)	n	USA	Shoemaker and Wyllie-Echeverria 2013
un(6)	Macroalgae (11/25)	n	India	Suryanarayanan et al. 2010
Me, Rh	Macroalgae (2/9)	n	Shetland	Flewelling et al. 2013
Ca, De	Macroalgae (un)	f	Japan	Uchida and Murata 2004
Ca, Me	Alga (1)	d	USA	van Uden and Castelo-Branco 1963
Ca(2), Rh(2)	Macroalgae (9)	n	USA	Seshadri and Sieburth 1971
Ca(2), Sp	Seagrass (3)	n	Bermuda	Wilson 1998
Ca, Cr, Rh(2)	Algae (8), seagrass (2)	n	USA	Roth et al. 1962
Ca(19), Pi, Rh(6)	Macroalgae (9)	n	USA	Seshadri and Sieburth 1975
Ca(2), Sa, Pi, To(2)	Phycosphere water (un)	n	USA	van Uden and Zobell 1962
Au?, Ca, Rh, Tr?	Macroalgae (2)	n	USA	Phillips 1982
Au, Cr, Me, Rh, un	Macroalgae (3)	n	Antarctica	Loque et al. 2010
Ca, Cr, Rh, Sa(2),To(2)	Macroalgae (5)	n	India	Patel 1975
Ca(2), Rh, To, Tr(3)	Macroalgae (9/24)	d	Japan	Suehiro 1960
Ca(5), Cr(4), Rh, To, Tr(2)	Macroalgae (7)	d	Japan	Suehiro and Tomiyazu 1962
Ca, Cr(2), De, Me(2), My, Rh, Ya	Macroalgae (8)	d	Antarctica	Godinho et al. 2013

Chung and Lee 2008, Lam et al. 2008) on macrophytes. The extraordinary species diversity and variety of interactions of protists in the sea are only slowly being appreciated (Sherr et al. 2007).

Therefore, there is a lack of information on distribution of many of those protists associated with marine hosts. It is suspected that some of these organisms may be common parasites in many marine ecosystems worldwide; however, at present there are only anecdotal data available to support this hypothesis (Neuhauser et al. 2011). It is surprising that only few studies have considered the abundance of heterotrophic protists on macrophytes as macroalgae (Armstrong et al. 2000). Molecular approaches have demonstrated that poorly recognized groups can be important ecosystem components (Richards et al. 2012), and parasites are increasingly being considered to be equally important to predators for the functioning and the stability of these important coastal ecosystems (Gachon et al. 2010, Li et al. 2010). Beyond characterizing the diversity and distribution of protists in the ocean, major lines of research continue to elucidate the ecological roles of protists in marine ecosystems, i.e., food webs, pathogens, degraders (Sherr et al. 2007). More research on these microbes, their life cycle, abundance and

distribution is needed to reliably estimate the impacts that these associations might have (Goecke et al. 2012b).

Conclusions and future perspectives

Macrophytes provide suitable microniches for microbes more favorable than in free water. They can act as an important reservoir for diverse microbes during adverse conditions (van Uten and Castelo Branco 1963, Englebert et al. 2008, Barott et al. 2011). The pathogenic role of marine microbes, although dispersed, has been corroborated (e.g., Andrews 1976, Apt 1988, Gachon et al. 2010) however, the presence and relevance of symbiotic microbial communities in marine macrophytes remain unexplored. Particularly understudied is the role of epibiotic biofilms for infection and disease of the host. Do biofilms repel host-pathogens and parasites and if so, when and how? What are the conditions under which biofilms switch from beneficial or neutral to adverse or even toxic effects upon the host (Wahl et al. 2012). It remains to be seen how changes in environmental conditions such as increased eutrophication and elevated sea surface temperature influence the microbial communities associated with macrophytes (as Case et al. 2011, Fernandes et al. 2012) and how these changes affect the physiology and success of macrophytes around the world (Barott et al. 2011). Characterization of the microbiota closely associated with macrophytes is therefore a first step that may provide further insights into the complex interactions between microbes and macrophytes (Garcias-Bonet et al. 2012). We have to extend our knowledge of 'normal' microbial communities associated with macrophytes, what are the reasons for such different associations, and which ecological functions are these microbes responsible for. For example, bacterial endophytes are crucial for the survival of many terrestrial plants, but little is known about the presence and importance of endophytes of marine plants (Garcias-Bonet et al. 2012). Moreover, coastal ecosystems and in particular seagrass meadows, are currently declining at an alarming rate worldwide, leading to loss of biodiversity. Unfortunately, extensive and extremely high costing restoration efforts have had little success so far. Recent findings indicate that restoration efforts should not only focus on environmental stressors (e.g., eutrophication, sediment runoff and high salinity) as a cause of decline but should also consider internal ecological interactions, such as the presence and vigor of symbiotic or mutualistic relations (Campbell et al. 2011, van der Heide et al. 2012).

Marine macrophyte survival, growth, and reproduction are known to vary with numerous climatically-sensitive environmental variables, and there is mounting evidence that acidification or warming will negatively impact macrophytes by facilitating microbial infections (Campbell et al. 2011, Case et al. 2011, Fernandes et al. 2012). Although seaweeds are known to be vulnerable to physical and chemical changes in the marine environment, the impacts of ongoing and future climate change in seaweed-dominated ecosystems remain poorly understood (Harley et al. 2012). Schulz et al. (2002) hypothesized that the fungal endophyte-plant host interaction is characterized by a finely tuned equilibrium between fungal virulence and plant defense, and if this balance is disturbed by either a decrease in plant defense or an increase in fungal virulence, disease can develop. Not only is the physiological state

of the host/microbes affected but also connectivity among host and potential vectors. Today there is a consensus that the recent shift towards intensive algal aquaculture and production methods correlates with more damaging disease outbreaks (Gachon et al. 2010). For example, macroalgae with buoyant structures which have a high floating potential, continue to grow after detachment and persist in a floating condition for long time periods. Depending on the prevailing winds and currents, macrophytes can be common dispersal vehicles for associated benthic invertebrates (Wichmann et al. 2012, and references therein). Microbes are transported as well (Thiel and Gutow 2005). Thus, this has been postulated as one route to transfer pathogens and symbionts among macrophytes (Meusnier et al. 2001, Hollants et al. 2013a, Aires et al. 2013). For example, roots of *Zostera* sp. infected with the phytomyxid *Plasmodiophora bicaudata* produce poorly developed roots and uprooting takes place relatively easily, releasing many floating infected *Zostera* plants (den Hartog 1989). This theory can be corroborated with molecular methods and experiments on survival and infective potential.

Kelp forests and seagrass meadows worldwide are known as hotspots for macroscopic biodiversity and primary production, yet very little is known about the biodiversity and roles of microorganisms in these ecosystems (Bengtsson et al. 2012). Macrophytes are important foundation species as they can modify their abiotic environment, e.g., reduce hydrodynamic energy from currents and waves, increase sediment accretion, alter sediment quality and stabilize sediments (Bos et al. 2007, Venier et al. 2012, and references therein). Such foundation species may enhance diversity by facilitating the presence of other organisms or communities which may eventually lead to succession (Bouma et al. 2009). Macrophytes serve as feeding, breeding and nursery grounds for economically important marine organisms including endangered species such as some fishes, mollusks and marine turtles (Sakayaroj et al. 2010). Microbes are benefited and also sustained by macrophytes. Through full and partial degradation of dissolved and particulate organic carbon, microbes make macrophyte primary production available to many animal consumers (Bengtsson et al. 2012). Microorganisms are an intrinsic part of those biotic environments, and in fact those microbes can be the real reason of ecological success of macrophytes in certain environments. Marine macrophytes have been challenged throughout their evolution by microorganisms and have developed in a world of microbes (Goecke et al. 2010), and during the last two decades exciting new studies have opened the door to understand such invisible but often vital connections in coastal ecosystems.

References

Aires, T., E.A. Serrao, G. Kendrick, C.M. Duarte and S. Arnaud-Haond. 2013. Invasion is a community affair: Clandestine followers in the bacterial community associated to green algae, *Caulerpa racemosa*, track the invasion source. PLoS ONE 8: e68429.

Almaraz, T., J. Checa and T. Gallardo. 1994. Ascomycetes asociados a *Pelvetia canaliculata* Decne. and Thur. (Fucales, Phaeophyta). Anales Jard. Bot. Madrid 52: 3–6.

Alva, P., E.H.C. McKenzie, S.B. Pointing, R. Pena-Muralla and K.D. Hyde. 2002. Do sea grasses harbour endophytes? Fungal Divers 7: 167–178.

Amin, S.A., D.H. Green, M.C. Hart, F.C. Kupper, W.G. Sunda and C.J. Carrano. 2009. Photolysis of iron-siderophore chelates promotes bacterial-algal mutualism. Proc. Natl. Acad. Sci. U.S.A. 106: 17071–17076.

Ando, Y., T. Nakagawa, R. Takahashi, K. Yoshihara and T. Tokuyama. 2009. Seasonal changes in abundance of ammonia-oxidizing Archaea and ammonia-oxidizing bacteria and their nitrification in sand of an eelgrass zone. Microbes Environ. 24: 21–27.

Andrews, J.H. 1976. The pathology of marine algae. Biol. Rev. Camb. Philos. Soc. 51: 211–253.

Apt, K. 1988. Galls and tumor-like growths on marine macroalgae. Dis. Aquat. Organ. 4: 211–217.

Armstrong, E., A. Rogerson and J.W. Leftley. 2000. The abundance of heterotrophic protists associated with intertidal seaweeds. Estuar. Coast Shelf Sci. 50: 415–424.

Armstrong, E., L.M. Yan, K.G. Boyd, P.C. Wright and J.G. Burgess. 2001. The symbiotic role of marine microbes on living surfaces. Hydrobiologia 461: 37–40.

Bagwell, C.E., J.R. La Rocque, G.W. Smith, S.W. Polson, M.J. Friez, J.W. Longshore and C.R. Lovell. 2002. Molecular diversity of diazotrophs in oligotrophic tropical seagrass bed communities. FEMS Microb. Ecol. 39: 113–119.

Balakirev, E.S., T.N. Krupnova and F.J. Ayala. 2012. Symbiotic associations in the phenotypically-diverse brown alga *Saccharina japonica* Plos One 7: e39587.

Ballesteros, E., D. Martin and M.J. Uriz. 1992. Biological activity of extracts from some Mediterranean macrophytes. Bot. Mar. 35: 481–485.

Barott, K.L., B. Rodriguez-Brito, J. Janouskovec, K.L. Marhaver, J.E. Smith, P. Keeling and F.L. Rohwer. 2011. Microbial diversity associated with four functional groups of benthic reef algae and the reef-building coral *Montastraea annularis*. Environ. Microbiol. 13: 1192–1204.

Bengtsson, M.M., K. Sjøtun and L. Øvreås. 2010. Seasonal dynamics of bacterial biofilms on kelp (*Laminaria hyperborea*). Aquat. Microb. Ecol. 60: 71–83.

Bengtsson, M.M., K. Sjøtun, J.E. Storesund and L. Øvreås. 2011. Utilization of kelp-derived carbon sources by kelp surface associated bacteria. Aquat. Microb. Ecol. 62: 191–199.

Bengtsson, M.M., K. Sjøtun, A. Lanzén and L. Øvreås. 2012. Bacterial diversity in relation to secondary production and succession on surfaces of the kelp *Laminaria hyperborea*. ISME J. 6: 2188–2198.

Bergmann, N., B. Fricke, M.C. Schmidt, V. Tams, K. Beining, H. Schwitte, A.A. Boettcher, D.L. Martin, A.C. Bockelmann, T.B. Reusch and G. Rauch. 2011. A quantitative real-time polymerase chain reaction assay for the seagrass pathogen *Labyrinthula zosterae*. Mol. Ecol. Resour. 11: 1076–1081.

Bos, A.R., T.J. Bouma, G.L.J. de Kort and M.M. van Katwijk. 2007. Ecosystem engineering by annual intertidal seagrass beds: Sediment accretion and modification. Estuar. Coast. Shelf Sci. 74: 344–348.

Bouma, T.J., V. Ortells and T. Ysebaert. 2009. Comparing biodiversity effects among ecosystem engineers of contrasting strength: Macrofauna diversity in *Zostera noltii* and *Spartina anglica* vegetations. Helgol. Mar. Res. 63: 3–18.

Bunt, J.S. 1955. The importance of bacteria and other microorganisms in the seawater at Macquarie Island. Aust. J. Mar. Fresh. Res. 6: 60–65.

Burke, C., P. Steinberg, D. Rusch, S. Kjelleberg and T. Thomas. 2011a. Bacterial community assembly based on functional genes rather than species. Proc. Natl. Acad. Sci. U.S.A. 108: 14288–14293.

Burke, C., T. Thomas, M. Lewis, P. Steinberg and S. Kjelleberg. 2011b. Composition, uniqueness and variability of the epiphytic bacterial community of the green alga *Ulva australis*. ISME J. 5: 590–600.

Campbell, A.H., T. Harder, S. Nielsen, S. Kjelleberg and P.D. Steinberg. 2011. Climate change and disease: Bleaching of a chemically defended seaweed. Glob. Chang. Biol. 17: 2958–2970.

Case, R.J., S.R. Longford, A.H. Campbell, A. Low, N.A. Tujula, P.D. Steinberg and S. Kjelleberg. 2011. Temperature induced bacterial virulence and bleaching disease in a chemically defended marine macroalga. Environ. Microbiol. 13: 529–537.

Chemello, R. and M. Milazzo. 2002. Effect of algal architecture on associated fauna: Some evidence from phytal molluscs. Mar. Biol. 140: 981–990.

Choi, H.G., J.H. Lee, H.H. Park and F.A.Q. Sayegh. 2009. Antioxidant and antimicrobial activity of *Zostera marina* L. extract. Algae 24: 179–184.

Chung, M.H. and K.S. Lee. 2008. Species composition of the epiphytic diatoms on the leaf tissues of three *Zostera* species distributed on the Southern Coast of Korea. Algae 23: 75–81.

Cifuentes, A., J. Antón, S. Benlloch, A. Donnelly, R.A. Herbert and F. Rodríguez-Valera. 2000. Prokaryotic diversity in *Zostera noltii*-colonized marine sediments. Appl. Environ. Microbiol. 66: 1715–1719.

Cifuentes, A., J. Antón, R. De Wit and F. Rodríguez-Valera. 2003. Diversity of Bacteria and Archaea in sulphate-reducing enrichment cultures inoculated from serial dilution of *Zostera noltii* rhizosphere samples. Environ. Microbiol. 5: 754–764.

Crump, B.C. and E.W. Koch. 2008. Attached bacterial populations shared by four species of aquatic angiosperms. Appl. Environ. Microbiol. 74: 5948–5957.

Cuomo, V., F. Vanzanella, F. Fresi, F. Cinelli and L. Mazzella. 1985. Fungal flora of *Posidonia oceanica* and its ecological significance. Trans. Br. Mycol. Soc. 84: 35–40.

Cuomo, V., E.B.G. Jones and S. Grasso. 1988. Occurrence and distribution of marine fungi along the Coast of the Mediterranean Sea. Prog. Oceanog. 21: 189–200.

de Oliveira, L.S., G.B. Gregoracci, G.G.Z. Silva, L.T. Salgado, G.A. Filho, M.A. Alves-Ferreira, R.C. Pereira and F.L. Thompson. 2012. Transcriptomic analysis of the red seaweed *Laurencia dendroidea* (Florideophyceae, Rhodophyta) and its microbiome. BMC Genomics 13: 487.

Dean, R.L. and J.H. Connell. 1987. Marine invertebrates in algal succession. III. Mechanisms linking habitat complexity with diversity. J. Exp. Mar. Biol. Ecol. 109: 249–273.

Debenay, J.P. and C.E. Payri. 2010. Epiphytic foraminiferal assemblages on macroalgae in reefal environments of New Caledonia. J. Foramin. Res. 40: 36–60.

Deckert, R.J. and D.J. Garbary. 2005. *Ascophyllum* and its symbionts. VI. Microscopic characterization of the symbiotum. Algae 20: 225–232.

DeLong, E.F. 2007. Microbial domains in the ocean: A lesson from the Archaea. Oceanography 20: 124–129.

Den Hartog, C. 1989. Distribution of *Plasmodiophora bicaudata*, a parasitic fungi on small *Zostera* species. Dis. Aquat. Org. 6: 227–229.

Devarajan, P.T., T.S. Suryanarayanan and V. Geetha. 2002. Endophytic fungi associated with the tropical seagrass *Halophila ovalis* (Hydrocharitaceae). Indian J. Mar. Sci. 31: 73–74.

Dobretsov, S., H.U. Dahms, T. Harder and P.Y. Qian. 2006. Allelochemical defense against epibiosis in the macroalga *Caulerpa racemosa* var. *turbinata*. Mar. Ecol. Prog. Ser. 318: 165–175.

Drouin, A., C.W. McKindsey and L.E. Johnson. 2011. Higher abundance and diversity in faunal assemblages with the invasion of *Codium fragile* ssp. *fragile* in eelgrass meadows. Mar. Ecol. Prog. Ser. 424: 105–117.

Duarte, C.M., M. Holmer and N. Marbá. 2005. Plant microbe-interactions in seagrass meadows. pp. 31–60. *In*: Kristensen, E., R. Haese and J. Kotska (eds.). Macro- and Microorganisms in Marine Sediments. Coastal and Estuarine Studies 60, American Geophysical Union, Washington.

Egan, S., T. Harder, C. Burke, P. Steinberg, S. Kjelleberg and T. Thomas. 2013. The seaweed holobiont: Understanding seaweed-bacteria interactions. FEMS Microbiol. Rev. 37: 462–476.

Eggert, A., A.F. Peters and F.C. Küpper. 2010. The potential impact of climate change on endophyte infections in kelp sporophytes. pp. 139–154. *In*: Israel, A., R. Einav and J. Seckbach (eds.). Seaweeds and their Role in Globally Changing Environments. Cellular Origin, Life in Extreme Habitats and Astrobiology 15. Springer.

Englebert, E.T., C. McDermott and G.T. Kleinheinz. 2008. Effects of the nuisance algae, *Cladophora*, on *Escherichia coli* at recreational beaches in Wisconsin. Sci. Total Environ. 404: 10–17.

Fernandes, N., P. Steinberg, D. Rusch, S. Kjelleberg and T. Thomas. 2012. Community structure and functional gene profile of bacteria on healthy and diseased thalli of the red seaweed *Delisea pulchra*. PLoS ONE 7: e50854.

Finster, K., T.R. Thomsen and N.B. Ramsing. 2001. *Desulfomusa hansenii* gen. nov., sp. nov., a novel marine propionate-degrading, sulfate-reducing bacterium isolated from *Zostera marina* roots. Int. J. Syst. Evol. Microbiol. 51: 2055–2061.

Flewelling, A.J., J.A. Johnson and C.A. Gray. 2013. Isolation and bioassay screening of fungal endophytes from North Atlantic marine macroalgae. Bot. Mar. 56: 287–297.

Friedrich, M.W. 2012. Bacterial communities on macroalgae. pp. 189–201. *In*: Wiencke, C. and K. Bischof (eds.). Seaweed Biology, Ecological Studies 219. Springer-Verlag, Berlin Heidelberg.

Gachon, C.M.M., T. Sime-Ngando, M. Strittmater, A. Chambouvet and G.H. Kim. 2010. Algal diseases: Spotlight on a black box. Trends Plant Sci. 15: 633–640.

Garcias-Bonet, N., T.D. Sherman, C.M. Duarte and N. Marbà. 2011. Distribution and pathogenicity of the protist *Labyrinthula* sp. in Western Mediterranean seagrass meadows. Estuar. Coast. 34: 1161–1168.

Garcias-Bonet, N., J.M. Arrieta, C.N. de Santana, C.M. Duarte and N. Marbà. 2012. Endophytic bacterial community of a Mediterranean marine angiosperm (*Posidonia oceanica*). Front. Microbio. 3: 342.

García-Martínez, M., J. Kuo, K. Kilminster, D. Walker, R. Rosselló-Mora and C.M. Duarte. 2005. Microbial colonization in the seagrass *Posidonia* spp. roots. Mar. Biol. Res. 1: 388–395.