

From the Sea to the Land: Dynamic of the Sargassum Tide Holobiont in the Caribbean Islands

Pascal Jean Lopez (✉ pascal-jean.lopez@mnhn.fr)

CNRS Délégation Paris B <https://orcid.org/0000-0002-9914-4252>

Vincent Hervé

Max-Planck-Institut for Terrestrial Microbiology

Josie Lambourdière

Centre National de la Recherche Scientifique

Malika René-Trouillefou

Universite des Antilles et de la Guyane

Damien Devault

Centre National de la Recherche Scientifique

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Abstract

Background

Over the last decade, intensity and frequency of *Sargassum* blooms in the Caribbean Sea and central Atlantic Ocean have dramatically increased, causing growing ecological, social and economic concern throughout the entire Caribbean region. These golden-brown tides form an ecosystem that maintains life for a large number of associated species, and their circulation across the Atlantic Ocean support the displacement and maybe the settlement of various species, especially microorganisms. To comprehensively identify the micro- and meiofauna associated to *Sargassum*, one hundred samples were collected during the 2018 tide events that were the largest ever recorded.

Results

We investigated the composition and the existence of specific species in three compartments, namely, *Sargassum* at tide sites, in the surrounding seawater, and in inland seaweed storage sites. Metabarcoding data revealed shifts between compartments in both prokaryotic and eukaryotic communities, and large differences for eukaryotes especially bryozoans, nematodes and ciliates. Among the most abundant nematodes, we identified various bacterivores in all compartments, suggesting trophic interactions in *Sargassum* tides. We also found a number of species of interest, including methanogenic archaea, sulfate- and nitrate-reducing bacteria as well as putative pathogens.

Conclusions

Here we present novel information on the diversity and trophic interactions of the micro- and meiofauna that are associated with *Sargassum* at tide and storage sites. Such information may help to better understand the ecological consequences of the *Sargassum* crises, and to develop proper analyses of the *Sargassum* associated biodiversity that could be important for application purposes such as biogas or fertilizers production, and to local authority for risks assessment.

Background

Numerous seaweeds, including the benthic and drifting pelagic *Sargassum* species, are becoming invasive worldwide, creating a potential threat to native species and local resources, and causing a number of economic and health concerns [1]. Over the last decade, the whole Caribbean region and the West coast of Africa were confronted with massive but irregular brown seaweed tides of the *Sargassum* complex [2]. These holopelagic macroalgae shoals are composed of two common species, namely, *S. fluitans* and *S. natans*, associated with rarer varieties. The huge biomass of seaweed washed up along coastlines has direct and indirect consequences on beaches and the functioning of near-shore ecosystems [3-5], and a massive influence on the tourist industry and local economies (*i.e.*, the cost of removing and disposing of piled up *Sargassum*, housing markets, etc.). *Sargassum* tides can also have a serious health impact, not only because of the toxicity of associated heavy metals and putative

pollutants, but also through their decomposition that can lead to the production of hydrogen sulfide (H₂S) and ammonia (NH₃), which are known to affect humans.

Drifting pelagic *Sargassum* in the Atlantic Ocean constitutes a floating ecosystem serving as a habitat for a number of species. Many of them might be adapted to such ecosystems, and a number of them correspond to endemic species, including Crustaceans, Teleostei, Cnidaria, Mollusca and Protostomia [6, 7]. Marine rafting organisms associated with *Sargassum* range from primary producers to top predators, and *Sargassum* serves as feeding and spawning habitats for marine species like sea turtles, seabirds, fish, invertebrates and micro- and macro-epiphytes [8-17]. Zooplankton which can accumulate near *Sargassum* might feed on periphyton and serve as prey for other pelagic organisms.

Members of the eukaryotic *Sargassum* epibionts have been described since the early 1970s and correspond to different phyla, including hydrozoans, bryozoans, polychaeta and blue-green algae. Other primary producers such as diatoms have been associated with *Sargassum* biofilms [18, 19]. Similarly, prokaryotic endophytic or epiphytic communities associated with marine macroalgae correspond to another fraction of their holobiont and are potentially involved in nutrient uptake, macroalgae spore release and germination, defense against bactericidal pathogens and other competing organisms, reproduction and settlement [20-22]. Exchanges between the holobiont and the surrounding environment have reciprocal impacts, and micro-organisms initially associated with algae growth might also contribute to their degradation processes [21]. More generally, microorganisms are likely to contribute to algae raft formation and maintenance, algae sinking and biodegradation upon beaching [23], and could provide important applications in the isolation of valuable compounds [24]. At molecular level, the bacterial diversity associated with genus *Sargassum* has been investigated in only a few locations [25-28], but neither the bacterial communities at tide sites nor the eukaryotic ones have ever been explored.

Our study aims to investigate the microbial community associated with *Sargassum* reaching or lying on the beaches along the coastline of two islands from the lesser Antilles, Guadeloupe and Martinique. We used a metabarcoding approach to describe both prokaryotic and eukaryotic diversity. Comparing sequence data from the seaweed-associated fractions to the contiguous waters, we attempted to identify specific OTUs associated with *Sargassum*. Finally, we also hypothesized that our analyses would provide novel information on species involved in algae biodegradation and putative exotic species associated with *Sargassum*.

Methods

Field sampling procedure. Samples were taken at beaching sites mainly along the Atlantic coasts of Martinique and Guadeloupe during the largest tidal events since 2011 (Fig. 1). Sampling was performed at the end of July and in early August 2018 at several beach locations. On most of the sites, the *Sargassum* seagrass was collected and the excess of seawater was drain-off using a salad spinner. We also collected the seawater surrounding the seagrass. Samples were either taken directly on the beach or in the water within a range of 0 to 20 m from the shore. As we took samples from various locations, the

color of the *Sargassum* varied from site to site, largely depending on the date of its arrival. However, since the macroalgae can rapidly decay when washed ashore, the marine seawater sampled was expected to have already been influenced by the presence of the seaweed. It was particularly visible in the brownish color of the water. In Martinique, we also took samples from four different sites used by the local collectivity to store the *Sargassum* that had been removed from the coast. All of the samples were put in sterile 50 ml Falcon tubes placed on ice and stored at -20 °C upon arrival at the laboratory until the nucleic acids had been extracted. Total environmental DNA was extracted from the filters of each sample using a PowerBiofilm DNA Isolation kit according to the manufacturer's recommendations (MO BIO, Qiagen, CA, USA).

DNA extraction and library preparation. The *Sargassum* samples were thawed at 4 °C and then suspended in 50 ml RNase-free H₂O (Millipore), vortexed and mechanically sonicated in a water bath (35 kHz) for 3 minutes. The latter procedure was repeated three times. Then the microbial fractions present in the supernatant and extracted from the seagrass were filtered through a 0.2 µm GTTP Biofilm Type Filter kit (Millipore). The water samples were filtered using the same method. The microbial DNA was extracted using the DNeasy PowerBiofilm Kit (Qiagen) according to the manufacturer's recommendations. The total DNA was then quantified with Quant-it PicoGreen (Invitrogen, Thermo Scientific Fischer Carlsbad, CA, USA) using a CFX96 Real-Time System, C1000 Touch Thermal Cycler Biorad (Biorad, California, USA).

The DNA libraries were prepared using universal primers for the 16S rRNA (16S-515F-Y rRNA: 5'-GTGYCAGCMGCCGCGGTAA-3', and 16S-926R: 5'-CCGYCAATYMTTTRAGTTT-3') or for the 18S rRNA (TAReuk454FWD1: 5'-CCAGCASCYGCGGTAATTCC-3', and TAReukREV3: 5'-ACTTTCGTTCTTGATYRA-3') genes. The DNA amplifications were performed in triplicate in a final volume of 25 µl following the Taq Q5 Hot Start High Fidelity DNA Polymerase recommendations (New England Biolabs). For the 16S rRNA amplification, we also added to the amplification reaction 0.4 µl of a 10 µM of peptide nucleic acid (PNA) clamps [29], specifically designed to match 16S RNA genes from *Sargassum* plastid (pPNA: 5'-AGCTCAACTTCAAAC-3') and mitochondria (mPNA: 5'-GGCTAGCCTTATTCGTC-3'). The PCR products were checked on agarose gels, purified using Agencourt AMPure XP beads (Beckman Coulter), and quantified using a Qubit dsDNA HS assay kit. The PCR products were then normalized and pooled (2 pools). The libraries were prepared using 1 µg DNA from the pools and the Illumina TruSeq Genomic Nano Library Preparation Kit. The supplier's protocols were followed, with the exception of the use of a modified End-Repair mix to avoid production of chimeric constructs, and no PCR cycle was done to finalize the libraries. The resulting libraries were quantified by qPCR and sequenced using a HiSeq 2500 2 × 300 paired-end run by Fasteris SA (Plan-les-Ouates, Switzerland).

Sequence processing. Amplicons of the 16S and 18S rRNA gene sequences were analyzed independently with the mothur software version 1.41.3 [30], following a standard operating procedure for Illumina MiSeq data [31]. First, contigs were assembled between the read pairs. Then the barcode, primer sequences and low-quality sequences were removed (minimum length of 350 bp and maximum length of 420 bp, removing any sequences with ambiguous bases and any sequences with homopolymers longer than

8 bp). The sequences were then aligned to the SILVA reference database release 132 [32] and preclustered (*pre.cluster*, *diffs* = 1). Singletons were excluded, and chimeras were removed with *vsearch* (Rognes *et al.*, 2016) implemented in *mothur*. The sequences were then classified using the k-nearest neighbor (*knn*) algorithm implemented in *mothur* and the BLASTN search method (cut-off of 80%) with the SILVA reference database release 132 and the PR2 database v4.11.1 [33] for the 16S and 18S rRNA gene amplicons, respectively. After classification, nonbacterial, chloroplast, mitochondria (for the 16S rRNA gene dataset), noneukaryotic, *Sargassum* (for the 18S rRNA gene dataset) and unknown (for both 16S and 18S rRNA amplicons) sequences were excluded. To account for differences in sampling efforts, 28,491 and 53,263 sequences from the 16S and 18S rRNA gene datasets respectively, were then randomly subsampled from each sample. Finally, operational taxonomic units (OTUs) were generated using the *vsearch* distance-based greedy clustering algorithm, with an OTU being defined at the 97% and 99% sequence similarity level for the 16S and 18S rRNA gene reads, respectively.

Diversity and statistical analyses. All the statistical analyses were computed using R software version 3.6.3. Principal coordinates analyses (PCoA) and constrained analysis of principal coordinates (CAP) were computed based on Bray-Curtis distances with the *phyloseq* package [34]. The effect of compartments on prokaryotic and eukaryotic community composition was tested by non-parametric permutational multivariate analysis of variance (PERMANOVA), as implemented in the *vegan* function *adonis* (permutations = 9999), after checking for homogeneity of group dispersions with the *vegan* function *betadisper* [35]. We also used permutation tests for CAP, as implemented in the *vegan* function *anova.cca* (permutations = 9999). To test the relationship between the bacterial and eukaryotic community matrices and between microbial community matrices and geographical location, partial Mantel tests were performed using the *ecodist* package [36], with Pearson correlation coefficient and 10^6 random permutations. To identify OTUs with differential abundances between substrate and medium, we used the ALDEx2 algorithm on the prokaryotic and eukaryotic community matrices after filtering out the OTUs with less than 0.01% abundance [37]. In this analysis, statistical significance was assessed based on the Wilcoxon rank test with Benjamini and Hochberg's correction to maintain a 5% false discovery rate. Functional inference of the prokaryotic OTUs was performed with FAPROTAX v1.1 [38]. FAPROTAX outputs were visualized with the *heatmap* R package, using Euclidean distances, and the weighted pair group method centroid (WPGMC) as a clustering method.

Phylogenetic analyses. Phylogenetic relationships between the abundant OTUs (> 1% relative abundance) of three clades, namely Nematoda, Armophorea and Plagiopylea, were investigated. For each clade, we first collected nearly full length 18S rRNA gene sequences from recent studies [39–41] as reference sequences. These sequences were aligned on the Silva SSU database using SINA v1.2.11 [42] and the subsequent alignments were filtered using *trimAl* v1.2rev59 with the *gappyout* method [43]. OTU sequences were added to the reference alignments using the *-addfragments* option of MAFFT v7.310 [44]. Alignments were trimmed and Smart Model Selection [45] was used to determine the best fit evolutionary model based on Akaike Information criterion. Subsequently, maximum-likelihood phylogenetic trees were built with PhyML 3.0 [46]. Branch supports were calculated using a Chi²-based

parametric approximate likelihood-ratio test (aLRT) [47]. Finally, each tree was visualized and edited with iTOL [48].

Results

Sampling strategies

In the present study, we investigated both prokaryotic and eukaryotic diversity associated with *Sargassum* during the 2018 tide events that were the largest ever recorded. We sampled the coastlines from different islands in the West Indies, corresponding to the Guadeloupe archipelago and the Martinique islands (Figure 1). One hundred samples were collected from the *Sargassum* seaweeds, the surrounding water of tide sites and *Sargassum* from inland storage sites. These three types of samples, or compartments, are hereafter named marine-*Sargassum* (M.Sarg), seawater (SW) and terrestrial-*Sargassum* (T.Sarg), respectively. In total, we obtained 22,214 prokaryotic and 17,679 eukaryotic operational taxonomic units (OTUs).

Community structure

Beta diversity of the *Sargassum* microbiota was first visualized using unconstrained ordinations, namely principal coordinates analyses (PCoA) (Additional file 1). PERMANOVAs indicated that both the prokaryotic ($R^2 = 0.08$, $P < 0.001$) and the eukaryotic ($R^2 = 0.09$, $P < 0.001$) communities were significantly different with regard to the three compartments M.Sarg, T.Sarg and SW. This pattern was further investigated using constrained ordinations, namely canonical analysis of principal coordinates (CAP) (Figure 2), and confirmed by permutation tests for CAP ($P < 0.001$). For both the prokaryotic and the eukaryotic communities, the first axis discriminated between M.Sarg and SW, while the second axis discriminated between T.Sarg and both M.Sarg and SW samples (Figure 2). Since a large fraction of variance remained unexplained by the factors investigated in this study, we also examined the relationship between the prokaryotic and eukaryotic dissimilarity matrices. A Mantel test revealed that the community structure of prokaryotes and eukaryotes were strongly correlated ($r_M = 0.43$, $P < 0.0001$), indicating potential biotic associations.

Diversity of *Sargassum*-associated communities

The overall prokaryotic diversity associated with the 100 *Sargassum*-related samples corresponded to 658 bacterial and archaeal families distributed across 350 orders, and 57 phyla. With 2.53% of the overall diversity, our results report the association between Archaea and *Sargassum* samples for the first time. When the diversity of the three different kinds of samples were analyzed independently (Figure 3A), we found that the most diverse phyla, which correspond to about 68% of the diversity, are, in decreasing order, the Proteobacteria, the Bacteroidetes and the Planctomycetes. Although we found slight differences for the terrestrial *Sargassum* samples, with an increase in the relative diversity of the Firmicutes, the 10

most represented orders were the same in all three compartments (Additional file 2). With regard to relative abundance (Figure 3A), the most abundant phyla were the Proteobacteria, followed by Bacteroidetes, Cyanobacteria, Spirochaetes and Firmicutes for the marine samples, while for the terrestrial ones they corresponded to Bacteroidetes, Proteobacteria, Planctomycetes, Firmicutes and Acidobacteria (Additional file 2). It should be noted that for the T.Sarg samples, Euryarchaeota phyla was the seventh most abundant one, with 2.08% of overall abundance.

The eukaryotic diversity spread across 278 orders, with an overall dominance of Opisthokonta in terms of both richness (24.9%) and read abundance (48.9%), followed by the Alveolata (22.3% of richness and 21.4% of abundance), and the Stramenopiles (20.7% of richness and 9% of abundance). The independent analysis of the three compartments revealed differences in both richness of species and abundance (Figure 3B). For the SW samples, the clades presenting the highest number of OTUs were the diatoms (Bacillariophyta), Alveolates corresponding to Dinophyceae, Syndiniales and Oligohymenophorea (Additional file 3). The SW samples' most abundant organisms corresponded to the Alveolata, Opisthokonta and photoautotrophs (Bacillariophyta, Florideophyceae, Ulvophyceae) (Additional file 3, panel A). Analyses of the most diverse clades for the M.Sarg samples showed a large diversity of Opisthokonta with five different phyla (Bryozoa, Arthropoda, Nematoda, Platyhelminthes and Cnidaria) among the twelve most diverse ones (Additional file 3, panel B). The difference in the diversity of the T.Sarg samples that was visible at the highest taxonomic level (Figure 3) was also clear at lower taxonomic ranks, with the Nematoda being the most dominant organisms, followed by Rhizaria and Amoebozoa-related OTUs (Additional file 3, panel C). The eukaryotic data revealed a large number of as yet unclassified eukaryotic OTUs in the different compartments. Focusing on the Opisthokonta, we also found strong differences between the compartments, with the Bryozoa dominating the M.Sarg samples, whereas Nematoda represented most of the diversity within the T.Sarg samples (Additional file 4). In fact, Bryozoa were extremely abundant on the surface of the collected *Sargassum* samples, especially on the M.Sarg ones, and could be observed macroscopically (Additional file 5). The taxonomy of the abundant Nematoda OTUs (> 1% relative abundance) was refined using phylogeny and putative trophic groups were inferred following Meldal et al. (2007) classification (Figure 4). We identified 24 OTUs belonging to 5 orders, namely Enoplida, Chromadorida, Monhysterida, Tylenchida and Rhabditida. Among the Monhysterida, we found ten OTUs including seven potentially bacterivore and particularly abundant in M.Sarg. On the contrary, the seven Tylenchida OTUs, all assigned to the genus *Halicephalobus*, and the four Rhabditida OTUs were mainly present in T.Sarg samples. Besides bacterivores, we also identified nematodes described as algivore-omnivore-predator, entomopathogen and vertebrate parasites.

Compositional analysis revealed *Sargassum*-specific associated OTUs

To analyze the differential abundance of OTUs between the three compartments, we applied the ALDEx2 algorithm. Regarding what we call here the “substrate effect”, which is the comparison between the surrounding seawater and the marine *Sargassum per se*, we identified 68 prokaryotic and 30 eukaryotic

OTUs with differential abundance (Figure 5A-B). For the prokaryotes, the surrounding seawater harbored 47 OTUs that were significantly more abundant than in the *Sargassum* samples, including three archaeal OTUs belonging to the Thermoplasmata Marine Group II, an abundant *Cryomorphaceae* OTU, two abundant members of the Synechococcaceae, a member of the Marinimicrobia clades, and six members of the SAR116 clade group of marine bacterioplanktons (Figure 5A, blue bars). On the other hand, compared to the seawater, the 21 OTUs found to be preferentially associated with the *Sargassum* samples corresponded to Cyanobacteria, Saprospiraceae, or one Planctomycetes clade OM190 (Figure 5A, brown bars). In the case of the eukaryotes, the 20 OTUs that were significantly associated with the surrounding seawater corresponded to Dinophyceae, Chlorophyta Cryptophyceae, Prymnesiales, Bacillariophyceae, one of the Demospongiae class, one Teleostean fish (probably from the Serranidae family), and one from the Thraustochytriaceae family (heterotrophic fungus-like clade of Labyrinthulomycetes) (Figure 5B, blue bars). The 10 OTUs that were significantly more abundant in *Sargassum* included Bryozoans, Maxillopoda, Dinoflagellata (one Syndiniophyceae and one Dinophyceae), one Hydrozoa of the genus *Zanclaea*, and one flatworm of the Rhabdocoela order (Figure 5B, brown bars).

Regarding the “medium effect”, corresponding to *Sargassum* collected on the seashore compared to *Sargassum* from inland storage sites, we identified 146 prokaryotic and 32 eukaryotic OTUs with differential abundance (Figure 5C-D). *Sargassum* samples from the beach showed significant enrichment of 93 OTUs prokaryotic OTUs. They contained Saprospiraceae, Flavobacteriaceae, Cyanobacteria, Alphaproteobacteria and Gammaproteobacteria, including the most abundant OTU in our dataset which was assigned to the genus *Vibrio*, and Planctomycetes (Figure 5C, brown bars). The prokaryotes found to be enriched in terrestrial samples represented 53 OTUs distributed over 9 phyla (Figure 5C, green bars). For the eukaryotes, the marine *Sargassum* showed significant enrichment in 24 OTUs, Dynophyceae, with some members probably from the genus *Amphidinium*, Bryozoans, Hydrozoans, Bacillariophyceae, a ciliate from the Stichotrichia subclass, and a Pelagophyceae likely to be the *Sargassococcus simulans* species (Figure 5D, brown bars). The *Sargassum* samples from storage sites showed significant enrichment of 8 OTUs, including a flagellated protist of the Colpodellidae order, a nematode that was assigned to the Monhysterida order, a filose amoeba, and one flagellate protist that was included in the Heterokonts (Figure 5D, green bars).

Functional inference of prokaryotic diversity

In order to investigate the microbial functions associated with the *Sargassum*-associated samples, we used FAPROTAX to predict the metabolic functions of the prokaryotic taxa and identified 2,831 prokaryotic OTUs (12.7 % of the global dataset) that could be functionally assigned to a minimum set of 22 functional groups (Figure 6). The five most abundant groups corresponded to chemoheterotrophy (1,260 OTUs; 23.19% of the abundance), fermentation (314 OTUs; 13.04% of the abundance), nitrate reduction (71 OTUs; 9.32% of the abundance), phototrophy (388 OTUs; 6.98% of the abundance), and respiration of sulfur compounds (404 OTUs; 3.91% of the abundance) (Figure 6). Intracellular parasites,

predators or exoparasites, and animal parasites or symbionts, corresponded to a relatively large number of organisms with 491, 263 and 189 OTUs, respectively. Interestingly, other functional groups were specifically abundant in terrestrial samples such as methanogenesis (32 OTUs; 0.21%), hydrocarbon degradation (21 OTUs; 0.31%) and dark oxidation of sulfur compounds (20 OTUs; 0.09%) (Additional file 6).

Discussion

Molecular studies of the microbial communities associated with *Sargassum* are relatively limited [25-28, 49] and to our knowledge absent from near shore Atlantic coasts. Here, we uncover both the prokaryotes and the eukaryotic communities associated with 100 samples corresponding to *Sargassum* seaweeds from tide sites, the seawater surrounding stranded *Sargassum* or near-shore rafts, and to *Sargassum* seaweeds from inland storage sites.

Specific prokaryotic diversity associated with *Sargassum* samples

The prokaryotic communities associated with the *Sargassum* tide-related samples were dominated by Proteobacteria (39.3%), Bacteroidetes (30.9%), Cyanobacteria (7.0%), Spirochaetes (5.5%) and Firmicutes (3.7%). Considering the three different compartments studied here, while the higher taxonomic ranking of diversity and abundance appeared quite similar (Figure 3A), some differences were visible in the order level (Additional file 2). The most abundant families associated with the marine *Sargassum* samples were the Vibrionaceae (Proteobacteria) (14.18%), a family of ubiquitous marine bacteria. Within the Flavobacteriaceae (11.23% of M.Sarg), we identified putative fish (*Flavobacterium*, 36 OTUs; *Tenacibaculum*, 3 OTUs) and human (*Elizabethkingia*, 4 OTUs) pathogens. The third one is the Saprospiraceae (Bacteroidetes) (8.14% of M.Sarg), a family that might contribute to the degradation of complex macromolecular release (*i.e.*, polysaccharides) from the seaweeds and their associated algae. The fourth family is the Spirochaetaceae (4.83% of M.Sarg), which was shown to dominate the microbial community associated with corals [50] and contained anaerobic species living in coastal marine sediments [51]. The next most abundant families were the Prolixibacteraceae (4.35% of M.Sarg), and the Rhodobacteraceae (2.88% of M.Sarg). Overall, our results show that the bacterial communities, which could be a mix of epiphytes and probably endophytes, associated with tide-associated *Sargassum*, are distinct from those in the surrounding seawater, confirming findings previously obtained for various macroalgae (for *Sargassum* sp. see [25, 26, 28]).

Interestingly, even with only nine samples of *Sargassum* collected from inland storage sites, striking differences were found at the highest taxonomy levels. In T.Sarg, Acidobacteria was the sixth most abundant phylum (4.06%), whereas these bacteria were barely present in the two other compartments analyzed (0.14 and 0.31% for the SW and M.Sarg, respectively). This phylum has been recognized as one of the most abundant and diverse on Earth, predominantly colonizing soil habitats [52]. The other striking

difference was for the FBP candidate division, with average abundance of 1.75% in T.Sarg and below 0.002% in the other samples. Members belonging to this taxon are known to have the capacity to degrade cellulose and hemicellulose [53] and are potential candidates for algae biomass degradation. At family level, we found the most abundant ones were the Flavobacteriaceae (9.31% of T.Sarg), the Prolixibacteraceae (5.36% of T.Sarg), and the Halomonadaceae (4.73% of T.Sarg). Interestingly, the two most abundant species correspond to the genera *Cobetia* and *Halomonas*. Some members of this genera were isolated in marine and coastal environments, but also in acid mine drainages which are environments with low pH, high salinity, toxic heavy metals and metalloids [54]. Interestingly, storage sites were shown to present high salinity levels and high concentrations of arsenic, which is bioaccumulated by *S. fluitans* and *S. natans* (Devault et al., unpublished data). The other most widely represented families were the Chitinophagaceae (4.21% of T.Sarg), the Pirellulaceae, which contain ammonia-oxidizing bacteria and bacteria associated with biofilms colonizing microplastics [55] and surface sediments, the Spirochaetaceae (3.57% of T.Sarg), and Acetobacteraceae (2.96% of T.Sarg). The most abundant OTU of the latter family was assigned to the genus *Acidiphilium*, containing aerobic bacteria producing acetic acid. Overall, our results highlight changes in prokaryotic diversity associated with the *Sargassum* holobiont upon terrestrial storage.

Metabolic predictions of *Sargassum*-associated microbiota

To gain insights into the roles of prokaryotic taxa, their putative functions were inferred for 2,831 OTUs. We found that 1,260 OTUs were deemed to be chemoheterotroph, representing 23.2% of the overall abundance (Figure 6). They belong to several bacterial genera, with the most abundant one being *Vibrio*, followed in M.Sarg by *Reinekea*, *Desulfomicrobium*, *Spirochaeta*, *Polaribacter*, *Cobetia* and *Alteromonadaceae*. Interestingly, the genus *Cobetia* harbors strains capable of crude oil degradation and producing biosurfactants [56], while *Reinekea* members were shown to use numerous molecular weight sugars and algal polysaccharides for growth, and are capable of oxidizing thiosulfate and fermenting under anoxic conditions [57]. The predominant bacteria predicted to perform nitrate reduction corresponds to *Vibrio*. Due to the complexity of the N cycle, with numerous possible substrates and product makes, it is difficult to assume the role of these bacteria in this instance, but they may make a significant contribution to N assimilation, either as free-living bacteria or associated with particles, zooplankton or other eukaryotes [58]. Phototrophy, corresponding mainly to abundant OTUs of the Oxyphotobacteria (Cyanobacteria) class, represented 241 OTUs and 6.9% of the total abundance. Cyanobacteria have long been described on the surface of *Sargassum* [59] and might also correspond to symbiotic association across the diverse prokaryotic and eukaryotic lineages present in *Sargassum* rafts.

When washed ashore, *Sargassum* will decompose and provoke significant environmental issues and health concerns due to the hydrogen sulfide (H₂S) and ammonia (NH₃) gas emissions. Functional prediction identified 404 OTUs involved in the respiration of sulfur compounds. These OTUs were assigned to several orders including Thermococcales (1 OTU), Clostridiales (51 OTUs), Desulfarculales (35 OTUs), Desulfobacterales (257 OTUs), Desulfovibrionales (87 OTUs), Desulfuromonadales (3 OTUs)

and Syntrophobacterales (3 OTUs). We also found OTUs that were not predicted by FAPROTAX but are likely to contribute to the S cycle. These corresponded to the genus *Sulfurovum* or *Sulfurimonas*. Some of these prokaryotes, which might originate from sediments on the marine surface layers, may contribute to *Sargassum* decomposition and hydrogen sulfide emissions. While preliminary studies of the Desulfobacterales family reveal a large number of putative novel species, understanding the diversity of sulfate-reducing microorganisms and sulfur-oxidizing microorganisms associated with the *Sargassum* decomposition process will require further study. In particular, investigation of the presence of functional S cycling-related genes (see, for example, Pelikan, 2016 #9032) will be needed. The sulfur cycle is linked to other cycles including carbon, metals and metalloids such as arsenic (As) I [60, 61]. This is of particular interest and another important issue given the large amount of As found in *S. natans* and *S. fluitans* along the coastlines of these islands (Devault et al., unpublished data).

A total of 491 OTUs were predicted to be symbiotic, with parasites or human and animal pathogens, including OTUs assigned to the Coxiellales order (128 OTUs of the genus *Coxiella*), Rickettsiales and Legionellales (55 OTUs of the genus *Legionella*). Members of these genera can cause severe disease in humans, and their presence in *Sargassum*-associated samples needs to be investigated with special care since they could well present potential risks. *Coxiella* were also shown to correspond to stable symbionts associated with corals [62]. The presence of prokaryotic symbionts and eukaryotic parasites can partially explain the significant correlation observed between prokaryotic and eukaryotic community structures.

In the terrestrial samples (T.Sarg), we found OTUs potentially involved in methanogenesis (Additional file 5) among the archaeal Methanomicrobiaceae, Methanosarcinaceae and Methanococcaceae families. These families are often members of microbial communities involved in methane production from algal biomasses [63]. Due to the anaerobic conditions associated with inland storage sites, especially those sampled which were essentially piles of *Sargassum*, our findings also suggest putative methanogenesis from *Sargassum* decomposition. This process could be of great interest in the biomass valorization of *Sargassum*.

Commonality and differences in eukaryotic diversity

Several taxonomic investigations devoted to specific classes or to the global eukaryotic communities have revealed the micro- and meiofauna associated with *Sargassum* spp. [19, 49, 64-67]. The present study reports the largest molecular inventory of eukaryotic species associated with *Sargassum*.

The largest group found corresponded to the opisthokonts, representing 24.9% of the total diversity and about half (48.9%) of the abundance. With a total of ~3,600 OTUs, our data highlight the significant diversity of metazoans, with considerable differences depending on the *Sargassum* samples (Additional file 4). With ~2,200 OTUs, the surrounding water was dominated, in decreasing order, by Arthropoda, Bryozoa and Annelida (Additional file 3). A contrasting result was found for the M.Sarg samples that were dominated by Bryozoa, Arthropoda, Platyhelminthes, Cnidarian and Nematodes (Additional file 3). Bryozoa, a large group of often calcified aquatic invertebrates [68], which is known to contain abundant

epibionts on *Sargassum* species [15, 66], were largely present on the surface of the collected *Sargassum* (Additional file 5). Phylogenetic analyses revealed that among the 490 OTUs assigned to Bryozoan, 438 of them were clustering with *Membranipora membranacea* (accession: JN680943.1) (not shown), including the most abundant OTU that largely dominates our dataset with 19.6% of the total abundance. Other previously described eukaryotes associated with *Sargassum* sp. were found such as Fungi (~410 OTUs; with putative endophytes of the genus *Corollospora* or *Arthrinium*) and Choanoflagellates (~190 OTUs) [64, 69].

Nematodes, which represented the most diverse group of Metazoans (21.4% of their total diversity), showed striking differences depending on the substrate type. They were largely dominant in terrestrial samples, with ~92% of the metazoan's abundance. The association between nematodes and floating *Sargassum* from the Atlantic region was already noted a century ago [70]. While colonization of such usually benthic meiofauna could be the result of accidental encounter, benthic nematode species were found attached to the surface of pelagic or benthic *Sargassum* spp [71-74]. In our dataset, we found that the most abundant OTUs could be present in the three different compartments, although with potentially specific patterns (Figure 4). Marine *Sargassum*-associated nematodes belong to the Enoplia and Monhysterida clades that have previously been described in marine habitats and present bacterivore and algivore-omnivore-predator trophic behaviors [39, 75]. Stranding and decaying stranded *Sargassum* along the shore could induce alterations in the structure of the intertidal soft-bottom and help nematode fauna to colonize the macroalgae. On the other hand, *Halicephalobus* (Tylenchina) and Rhabditidae, which are bacterivores [39], presented exclusive or specific patterns associated with terrestrial sites. Regarding the *Halicephalobus* OTUs, the best blast hit for Otu00067 and Otu00111 was *Halicephalobus mephisto* (accession number GQ918144), a bacterivore nematode found at the terrestrial deep subsurface that presented hypoxic conditions and a temperature of 37°C [76]. Similar conditions are expected to occur in T.Sarg samples.

After the Opisthokonts, the Alveolata were the second most diverse superphylum in our samples, including 1,932 dinophyceae and 1,557 ciliate OTUs, corresponding to 15.4% and 5.4% of the total abundance, respectively. Among the ciliates, the Oligohymenophorea, which corresponded to the third most abundant class, with 586 OTUs, were abundant in all sample types (Additional file 3). The Spirotrichea and Plagiopylea were especially present in the seawater samples and the Colpodea in the terrestrial ones (Additional file 3). This latter ciliophoran class is mainly present in terrestrial and semiterrestrial habitats, although there is some evidence of its presence in marine environment surveys [77, 78]. Among the Oligohymenophorea, some Scuticociliates are known to invade and colonize marine hosts' internal organs and can induce severe systemic infections. For instance, Scuticociliatosis has been reported in a broad range of teleosts [79-81]. Here, we found a number of OTUs which could be assigned to known causative agents of scuticociliatosis: *Pseudocohnilembus persalinus* or *P. longisetus* (37 OTUs were assigned to this genus), *Uronema marinum* (2 OTUs), and *Miamiensis avidus* (1 OTU), suggesting health concerns or at least surveillance.

Ciliates are versatile organisms and display considerable functional diversity, including in food webs, acting as predators of bacteria, algae, other protists and even some metazoans. Some ciliates are known to be sensitive to oxic/anoxic conditions [82-85] which can occur upon heavy accumulation of *Sargassum* on the shoreline. Ciliated protozoans were found to be abundant in mats dominated by sulfide-oxidizing bacteria [86], or in sulfur cycling in marine sediments [87]. Ciliates can also present chemosynthetic symbioses with bacteria and archaea [88], including endosymbionts that are involved in methane metabolism [89]. In particular, members of the Plagiopylea and Armophorea classes correspond to ciliates living in oxygen-depleted habitats, which have been shown to host endosymbiotic methanogens [40, 41, 90-93]. Here, we found a total of 134 OTUs belonging to these two classes of ciliates, with the most abundant OTUs present mainly in SW and M.Sarg samples (Supplementary Figs. 7-8). Our data suggest oxygen concentration depletion at least in marine tide sites, and that some of these organisms could be involved in methane and/or sulfate cycles along the shore.

The third most abundant supergroup, the Stramenopiles, was largely dominated by Bacillariophyceae, with ~2,000 OTUs or ~11% of the total diversity. After the diatoms came the Labyrinthulomycetes, which are protists found as parasites on unicellular phototrophs, green algae, and diatoms, and which present pathogenic behavior towards shell organisms (Additional file 3). More generally, heterotrophic protists are organisms commonly found on seaweed surfaces, and are known to play various roles, including in the consumption of organic materials such as dissolved organic carbon (DOC), released by seaweeds such as *Sargassum* [94]. Thus, the large diversity of protists found here are likely to make a significant contribution to primary and secondary production and to nutrient cycling in the surf and the swash zones [95].

Identification of *Sargassum* biomarkers

We used the ALDEx2 algorithm on the prokaryotic and eukaryotic community matrices to identify abundant OTUs with differential abundance between substrates (SW *versus* M.Sarg) and media (M.Sarg *versus* T.Sarg). Interestingly, the OTUs with differential abundance in the M.Sarg samples were Dinophyceae, three unclassified Maxillipoda, and probably copepods, which have often been described as associated with *Sargassum* sp. [66]; three OTUs corresponding to Bryozoa and probably to the genus *Membranipora* (see [15]), a hydrozoan of the genus *Zanclaea* that was described as being common on a hydroid assemblage on holopelagic *Sargassum* from the Sargasso Sea [65], and one OTU of flatworm of the Rhabdozoela order, some of whose species are known to live in association with *Sargassum* [96]. Among the 24 OTUs differentially expressed in the M.Sarg sites (Figure 5), somewhat remarkably, we found one OTU assigned to a toxic dinoflagellate of the *Amphidinium carterae* genus, another to *Ulvellia* that are endophytic microalgae, one related to the raphid diatom genus *Aneumastus*, one OTU with the best BLASTN results as *Sargassococcus simulans*, an epiphyte on floating *Sargassum thallus*, isolated in the Sargasso Sea, one to the copepod genus *Zaus*, which are organisms living in the phytal zone, and five to Hydrozoans (Opisthokonta, Cnidaria), which are also frequently encountered as *Sargassum* epibionts [66].

We also found 21 prokaryotic biomarkers in the M.Sarg samples, including cyanobacteria (4 OTUs) and Saprospiraceae (4 OTUs), which are important in the breakdown of complex organic compound (Figure 6). The M.Sarg samples only showed 4 OTUs that were differentially present in this compartment compared to T.Sarg which had two of the three most abundant OTUs in the whole dataset (a Cyanobiaceae and a Cryomorphaceae). In the terrestrial samples, 11 OTUs were considered as biomarkers, with Saprospiraceae (3 OTUs) and new environmental members of the Rivulariaceae family. Altogether, our data revealed a number of potential new molecular markers associated with *Sargassum* racks from the Caribbean, with a number of them corresponding to potentially new or poorly described species involved in the trophic food webs at tide sites.

Conclusions

Our study demonstrates specific micro- and meiofauna depending on the *Sargassum* samples and identified specific prokaryotic (archaea and bacteria) and eukaryotic biomarkers for marine waters at tide sites, near-shore and beach stranded wracks, and inland storage sites. The description of members of the meiofauna (*i.e.*, ciliates and nematodes) that may play important roles in determining community structures provides a firm grounding for further investigation of algal organic matter decomposition and nutrient transfers between the trophic levels, and emphasizes the need for careful investigation of the entire diversity that contributes to gas production. Based on our results, carefully designed molecular studies with the aim of delivering comparative data analyses can provide effective information on species dispersal via drifting *Sargassum*, and on biogeographical differences in the *Sargassum* holobiont.

Declarations

Availability of supporting data

The raw data sets generated and/or analyzed during the current study are available on the NCBI Sequence Read Archive (SRA) under the BioProjects PRJNA630532 (16S rRNA genes) and PRJNA630533 (18S rRNA genes).

Competing interests

The authors declare that they have no competing interests.

Electronic supplementary material

Additional file 1: Differences in community composition.

Additional file 2: Most diverse (*left*) and abundant (*right*) prokaryotic orders

Additional file 3: Most diverse (*left*) and abundant (*right*) eukaryotic clades.

Additional file 4: Richness and abundance within the Metazoa.

Additional file 5: Images showing bryozoa on *Sargassum*.

Additional file 6: Heatmap of predicted functions based on the FAPROTAX database.

Additional file 7: Phylogenetic tree of ciliates of the Plagiopylea class.

Additional file 8: Phylogenetic tree of ciliates from the SAL (Spirotrichea, Armophorea, Litostomatea) group.

Authors' contributions

Conceptualization, V.H. and P.J.L.; Methodology, V.H., J.L., M.R.T., D.D. and P.J.L.; Investigation, V.H. and P.J.L.; Writing – Original Draft, V.H., J.L. and P.J.L.; Funding Acquisition, P.J.L.

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Authors' information

Affiliations

Max Planck Institute for Terrestrial Microbiology, Karl-von-Frisch-Strasse 10, 35043 Marburg, Germany

Vincent Hervé

Laboratoire Biologie des ORganismes et Ecosystèmes Aquatiques (BOREA), Muséum National d'Histoire Naturelle, Centre National de la Recherche Scientifique, Sorbonne Université, Institut de Recherche pour le Développement, Université de Caen Normandie, Université des Antilles, 43 rue Cuvier 75005 Paris, France.

Josie Lambourdière, Malika René-Trouillefou, Damien Devault & Pascal Jean Lopez

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Figures

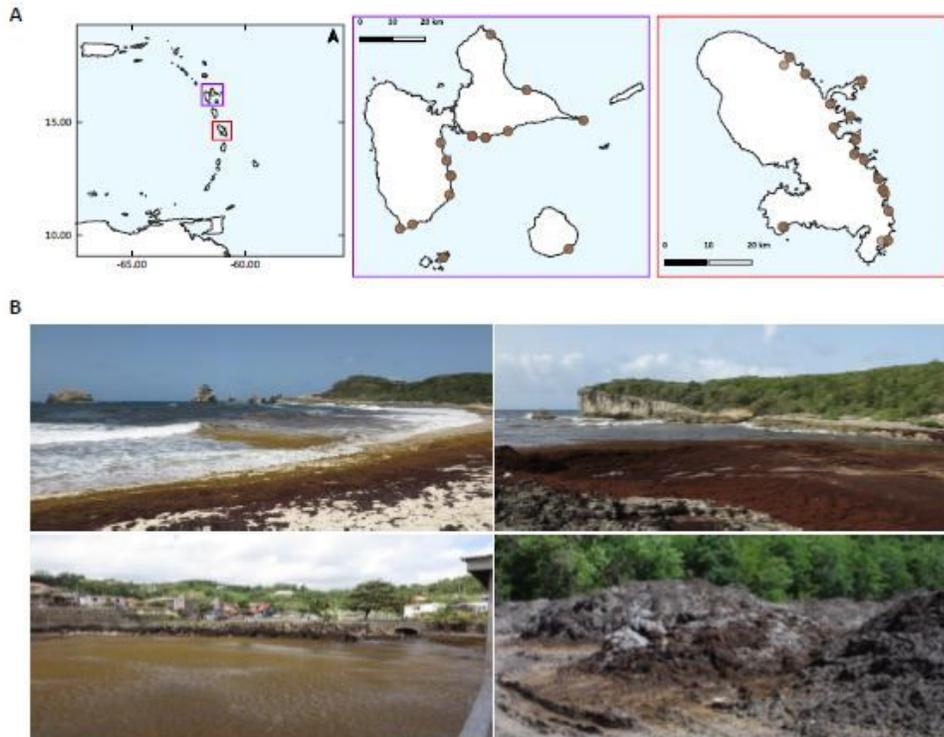


Figure 1: Maps of sampled *Sargassum* tide sites. The year 2018 corresponded to the biggest tide events ever recorded in the Caribbean region. (A) From left to right, the Caribbean Basin, the Guadeloupe archipelago (violet), and the Martinique island (red). Due to the proximity of the sampled sites, some circles may overlap. (B) Photographs taken during sampling revealing *Sargassum* accumulated along the shorelines and at one inland-storage site.

Figure 1

Maps of sampled *Sargassum* tide sites. The year 2018 corresponded to the biggest tide events ever recorded in the Caribbean region. (A) From left to right, the Caribbean Basin, the Guadeloupe archipelago (violet), and the Martinique island (red). Due to the proximity of the sampled sites, some circles may overlap. (B) Photographs taken during sampling showing *Sargassum* accumulated along the shorelines and at one inland-storage site.

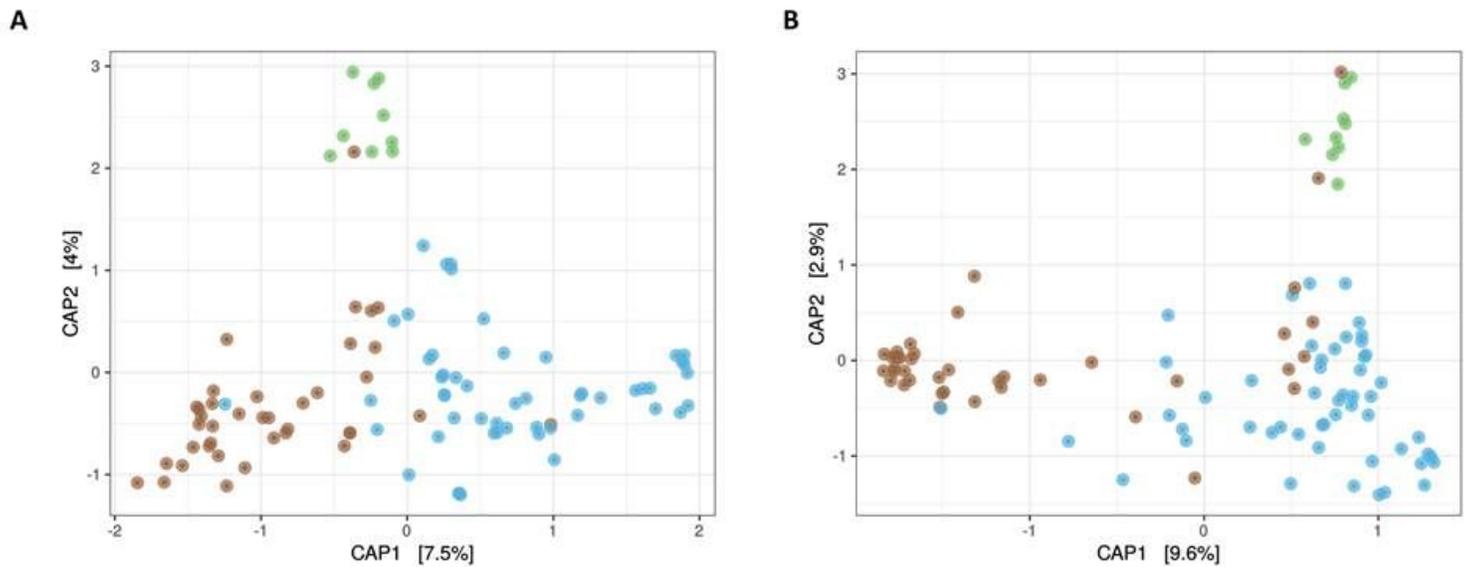


Figure 2

Differences in community composition. (A) Prokaryotic community and (B) Eukaryotic community, revealing significant differences (permutation tests for CAP, $P < 0.001$) between seawater (SW, in blue), marine Sargassum (M.Sarg, in brown), and terrestrial Sargassum (T.Sarg, in green).

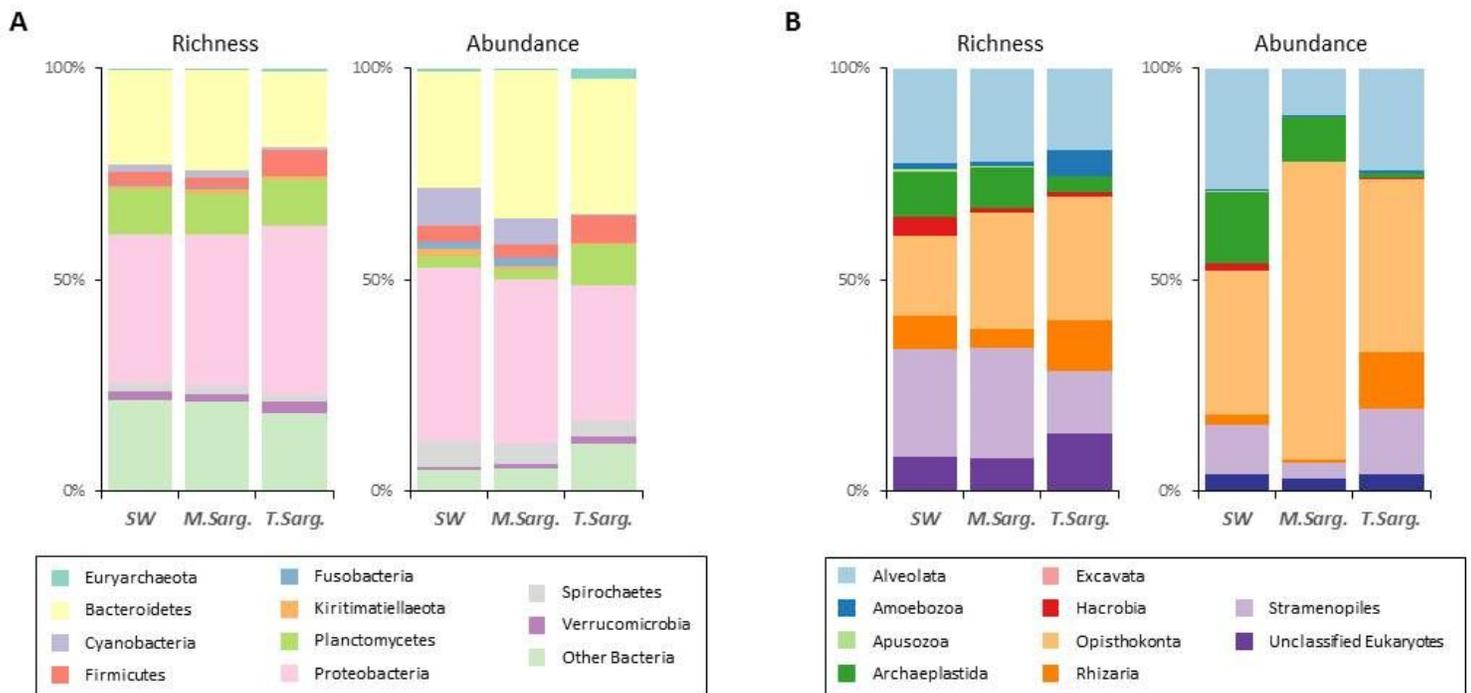


Figure 3

Molecular inventory of the biodiversity associated with Sargassum tides from 100 sampled sites. Differences in the richness and relative abundance of seawater (SW) surrounding marine Sargassum, marine Sargassum (M.Sarg) and terrestrial Sargassum (T.Sarg). (A) Prokaryotic diversity corresponds to

22,214 OTUs obtained from 2,849,100 reads. (B) Eukaryotic diversity corresponds to 17,679 OTUs obtained from 5,326,300 reads.

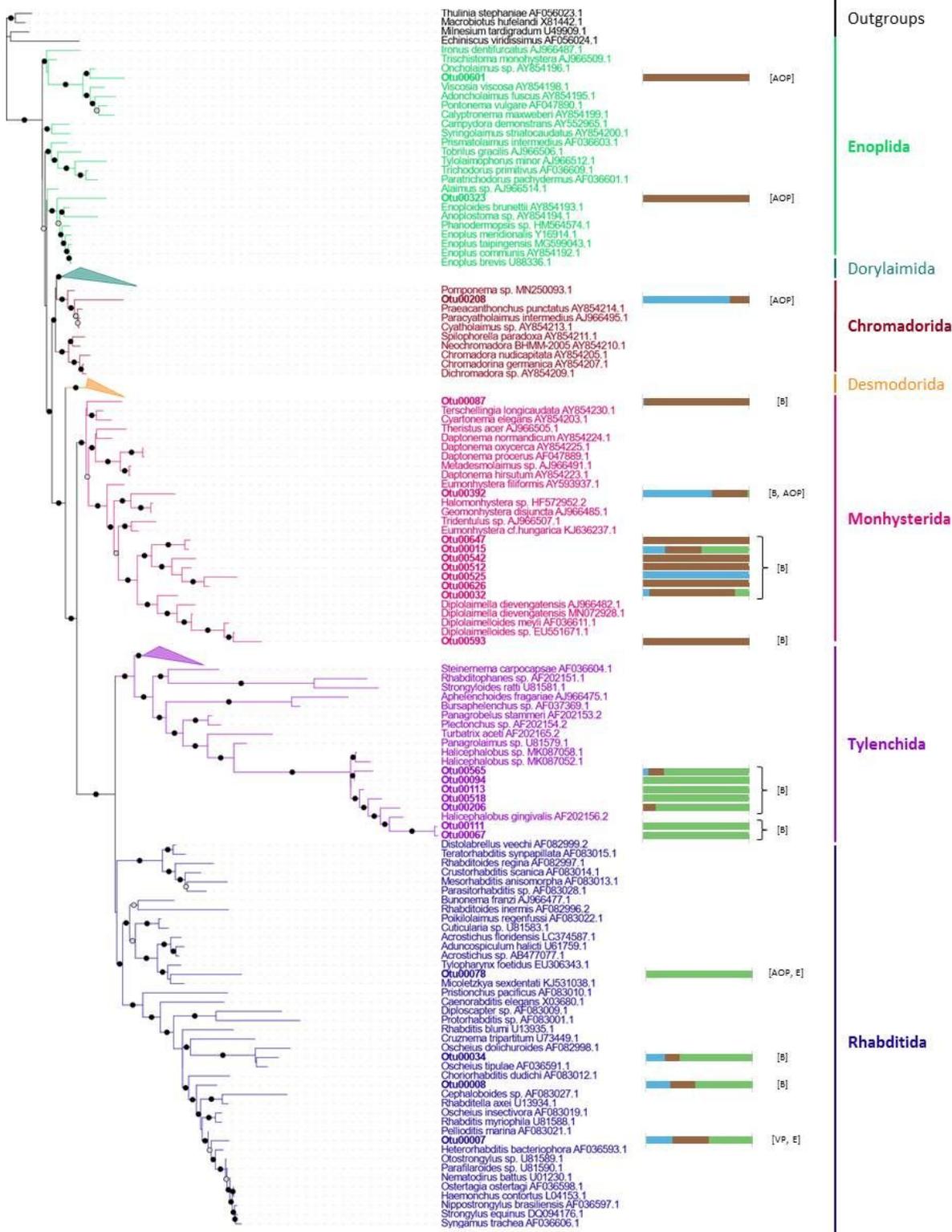


Figure 4

Phylogenetic relationships between the most abundant nematode OTUs associated with Sargassum. The maximum likelihood phylogenetic tree was constructed with PhyML v3.0 using the GTR+G+I model. Branch supports were calculated using a Chi2-based parametric approximate likelihood-ratio test and are

represented by black circles for values above 0.90 and gray circles for values between 0.7 and 0.89. OTU sequences are in bold. The horizontal bar plots represent the normalized total abundance of each OTU in the three compartments: SW in blue, M.Sarg in brown, and T.Sarg in green. Putative trophic groups were assigned following recommendations by Meldal et al. (2007): algivore-omnivore-predator [AOP], bacterivore [B], entomopathogen [E], and vertebrate parasite [VP].

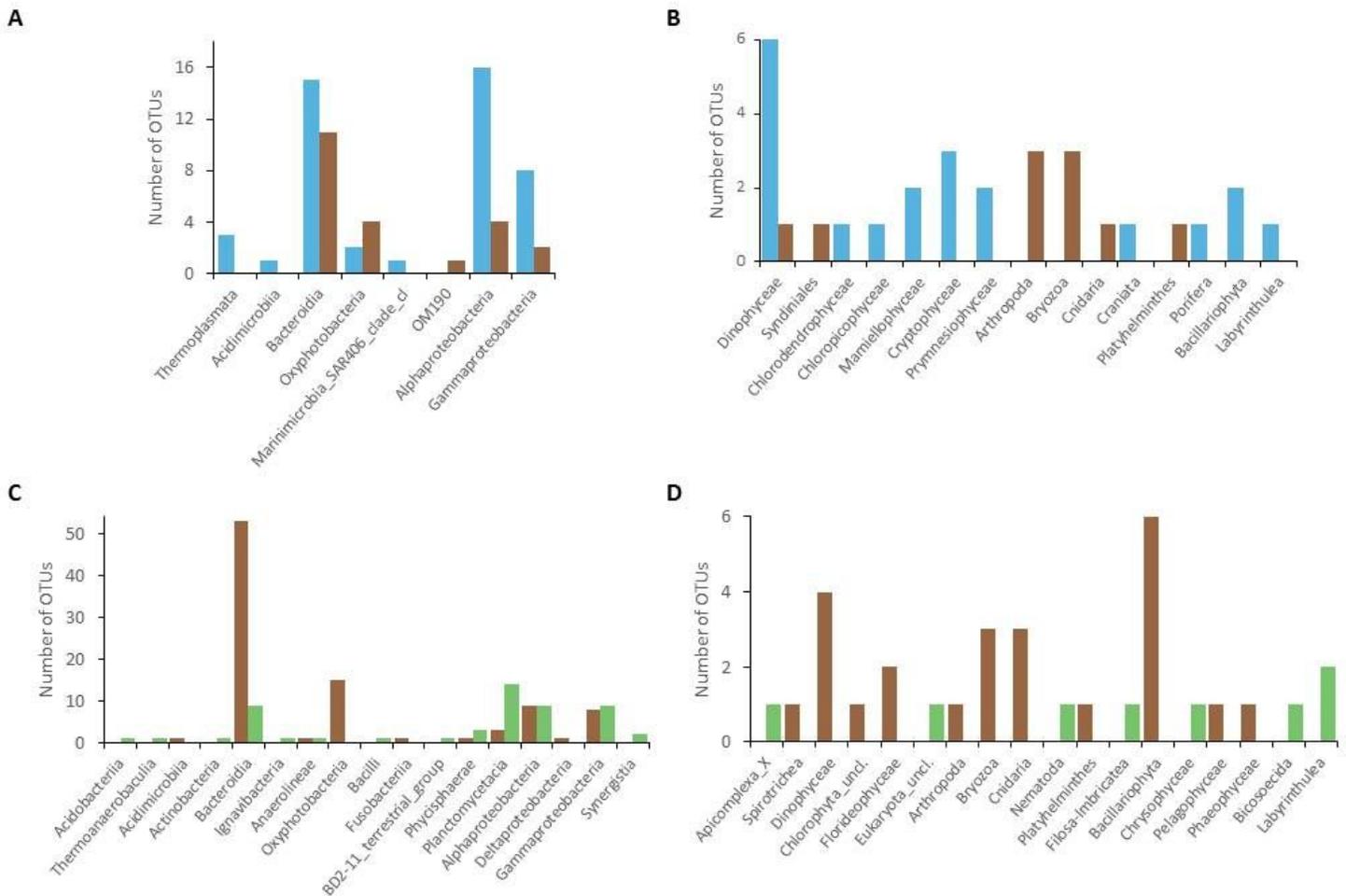


Figure 5

ANOVA-like differential expression (ALDEx) analysis of the OTUs presenting significant differential abundance for the different Sargassum-associated samples. OTUs that are differentially represented between seawater (blue) or marine Sargassum (brown): (A) Prokaryotic, and (B) Eukaryotic. OTUs that are differentially represented between marine Sargassum (brown) or terrestrial Sargassum (green): (C) Prokaryotic, and (D) Eukaryotic.

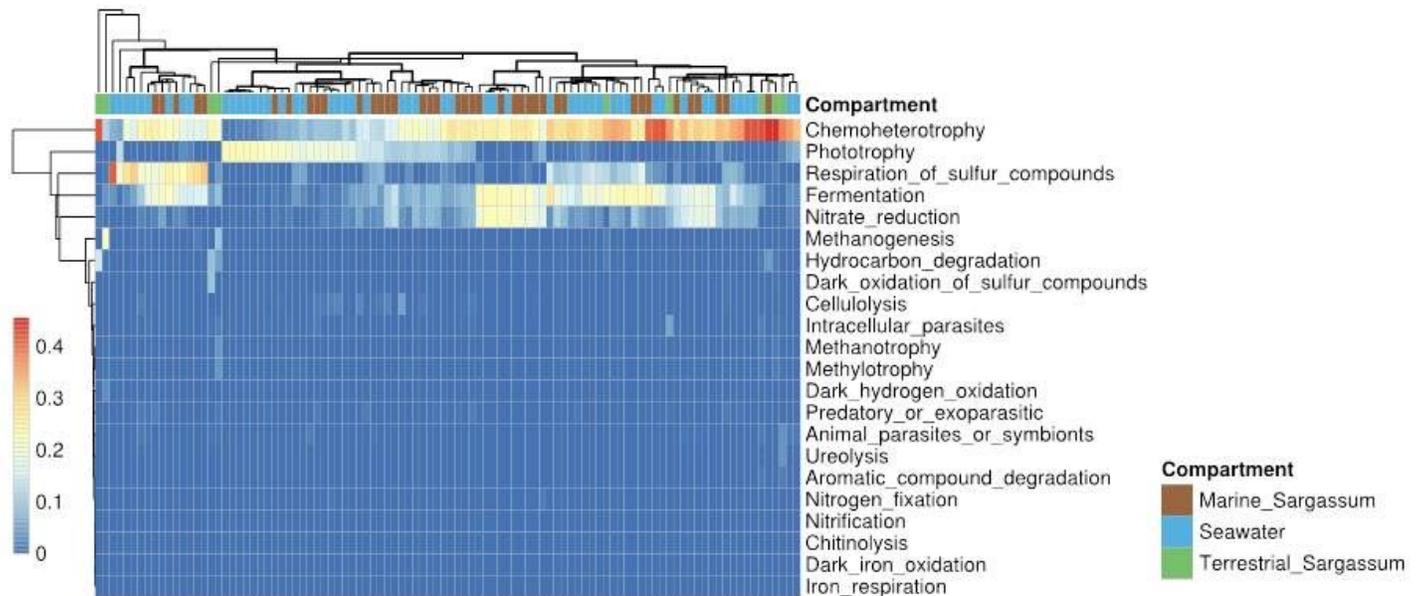


Figure 6

Heatmap of predicted functions based on the FAPROTAX database. The 22 categories presented here correspond to 2,831 prokaryotic OTUs. Data were clustered, with the weighted pair group method centroid (WPGMC) algorithm based on Euclidean distances. The color scale represents the proportion of each predicted function within a sample.

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