



Propagule pressure and native macrophyte biomass mediate the success of an invasive alga: the role of below-ground microbial communities

Chloe Renn · Sebastian Vadillo Gonzalez · Ezequiel M. Marzinelli · Katherine A. Dafforn · Josee Hart · Sofie E. Voerman · Louise B. Firth · Paul E. Gribben 

Received: 1 July 2024 / Accepted: 24 April 2025 / Published online: 20 May 2025
© The Author(s) 2025

Abstract Biological invasions pose a significant threat to local ecosystems and native species world-wide. For macrophytes, the interaction of factors such as native species biomass loss and propagule pressure of invasive species have important consequences for invasion success. How these factors are mediated by below-ground processes is, however, poorly understood. Through a field survey and manipulative experiment, we simultaneously investigated the influences of native seagrass (*Zostera muelleri*) biomass, invasive propagule pressure and below-ground bacterial communities (evaluated through 16S amplicon

sequencing) in mediating the establishment of the invasive green alga, *Caulerpa taxifolia*. Results from the field survey showed that rhizosphere bacterial communities and predicted metabolism were clearly distinct between *Z. muelleri* and *C. taxifolia*, but bulk sediment microbes and sediment characteristics (i.e. proportion of fine sediments and organic carbon) did not differ. Increasing seagrass or *C. taxifolia* biomass only affected rhizosphere bacterial richness, and this was apparent on specific bacterial groups involved in sulphur and nitrogen metabolism. Comparatively, the experimental manipulation showed that seagrass biomass significantly decreased *C. taxifolia* biomass, but this effect depended on invasive propagule levels. More specifically, *C. taxifolia* biomass was lower in treatments with higher *Z. muelleri* biomass although

Chloe Renn and Sebastian Vadillo Gonzalez contributed equally to this work and share first authorship.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10530-025-03599-0>.

C. Renn · L. B. Firth
School of Biological and Marine Sciences, University of Plymouth, Plymouth PL4 8AA, UK

S. Vadillo Gonzalez · E. M. Marzinelli
School of Life and Environmental Sciences, The University of Sydney, Sydney, NSW 2006, Australia

S. Vadillo Gonzalez · J. Hart · S. E. Voerman · P. E. Gribben (✉)
Centre for Marine Science and Innovation, School of Biological, Earth and Environmental Science, University of New South Wales, Sydney, NSW 2052, Australia
e-mail: p.gribben@unsw.edu.au

E. M. Marzinelli
Singapore Centre for Environmental Life Sciences Engineering, Nanyang Technical University, Singapore 637551, Singapore

K. A. Dafforn
School of Natural Sciences, Macquarie University, Sydney, NSW 2109, Australia

K. A. Dafforn
School for the Environment, University of Massachusetts Boston, Boston, MA 02125, USA

L. B. Firth
School of Biological, Earth and Environmental Sciences, University College Cork, Cork, Ireland

the magnitude of this effect was stronger in plots with high propagule pressure. Overall, increasing *C. taxifolia* propagule pressure may intensify negative interspecific competition among invading individuals, while higher seagrass biomass also reduces overall invasion success. These effects could potentially be mediated by root-associated microbial communities and directly influence invasion success.

Keywords *Caulerpa taxifolia* · Microbial community · Plant-soil feedbacks · Seagrass · Sediment · *Zostera muelleri*

Introduction

Biological invasions continue to pose a threat to global biodiversity, adversely impacting recipient communities and causing both ecological (e.g. Gribben et al. 2013; Gallardo et al. 2016; David et al. 2017) and economic harm (Ricciardi et al. 2011). Understanding the mechanisms behind invasion success is essential for conserving native biodiversity and ecosystems. Several theories addressing the success of invasive species in their introduced range have received empirical support, including niche shifts (changes in niche use or tolerance by invaders), trait shifts that enhance invader fitness and facilitate range expansion and environmental matching by invaders (reviewed in Gribben and Byers 2020). However, evidence for these theories is equivocal, differing amongst habitats and taxonomic groups (Jeschke et al. 2012), and possibly because such theories are mostly tested in isolation. There is growing evidence that invasion success is dependent on a range of interacting biological and environmental factors (Romanuk and Kolasa 2005; Leung and Mandrak 2007; Clark and Johnston 2009). Understanding these interactions may lead to better insight into the biological and/or environmental processes that promote invasion success and conversely provide biotic resistance to invasion.

Disturbance to native habitats and propagule pressure have well-documented effects on invasion success (Davis et al. 2000; Lockwood et al. 2013). For macrophytes, intact canopies formed by one or a few spatially dominant native foundation species can create resistance to invasion by reducing local resource availability, increasing competition, and by utilizing

their adaptation to local environmental conditions for more efficient resource use (Andrew and Viejo 1998; Scheibling and Gagnon 2006; Emery and Gross 2007; Salgado et al. 2022). Thus, a reduction in native species biomass or cover can create windows of opportunity driven by periodic fluctuations in resource availability (nutrients or space) or changes in community interactions, ultimately promoting a more favourable habitat for the establishment of invasive species (Davis et al. 2000; Lockwood et al. 2013). However, even at high canopy cover, resistance to invasion can be overwhelmed by high propagule pressure from the invader (Holle and Simberloff 2005; Hollebone and Hay 2007). Invasion success may also be facilitated at high levels of both disturbance (e.g. reduction of native species) and invader propagule supply (Clark and Johnston 2009). This suggests that interactions between disturbance and propagule pressure have important implications for invader success. To date, experimental manipulations of native foundation species (sensu Dayton 1972) typically focus on scenarios of high levels of disturbance, comparing the outcomes of complete loss or absence of native species with those of fully intact habitats (e.g. Prév  y et al. 2010; Guido and Pillar 2015). However, few studies have investigated how invasion success is determined across gradients of foundation species' biomass or cover loss, and how this may interact with propagule pressure.

Competition for above-ground resources such as light and space have been considered key drivers of plant invasion success both in terrestrial (e.g. Iponga et al. 2008; Gioria and Osborne 2014) and marine systems (Valentine and Johnson 2003; Valentine and Johnson 2004; Glasby 2013; but see Bulteri et al. 2018). Mounting evidence from terrestrial ecosystems demonstrates soil processes under microbial control can also determine the outcome of interactions between native and invasive plants (Callaway et al. 2004; van der Putten et al. 2007; Cipollini et al. 2012) in ways that benefit either the native or invasive competitor. For example, plant detritus or root exudates can promote the development of a soil microbial community that promotes nutrient cycling and chemical processes to support their own growth at expense of the competitor (Kourtev et al. 2003; Wolfe and Klironomos 2005; Elgersma et al. 2012). Disturbance to native plant soils can lead to changes in their microbial

community and shifts in soil nutrient cycling and chemical processes that benefit the establishment of the invader (Klironomos 2002; Fukano et al. 2013). While the role of below-ground processes in influencing invasive macrophyte success in soft-sediment marine ecosystems has received much less attention, evidence suggests that they may be as important as above-ground processes (Gribben et al. 2017, 2018; Bulleri et al. 2018, 2020).

Laboratory experiments show that active microbial communities in sediments from native seagrass *Zostera muelleri* and the invasive green alga *Caulerpa taxifolia* inhibited and promoted fragment success of the invader, respectively (Gribben et al. 2017). Field experiments have shown that the establishment of invasive *Caulerpa* spp. is higher with the complete removal of seagrass (both above and below-ground biomass) compared with the removal of only the canopy. This indicates that both sediment characteristics (e.g. organic matter content, Bulleri et al. 2018) and sediment biogeochemical processes, regulated by microbial communities, in native seagrass beds, play a crucial role in mediating invasion success (Gribben et al. 2018; Bulleri et al. 2020). Additionally, the effects of seagrass disturbance can interact with fragment morphological and physiological characteristics to determine establishment success (Bulleri et al. 2020), although how native seagrass biomass interacts with the supply of *C. taxifolia* propagules is unknown. The sediment chemistry and bacteria associated with *Caulerpa* spp. are indicative of more anaerobic environments compared to seagrasses (Holmer et al. 2009; McKinnon et al. 2009; Gribben et al. 2017). Rhizoid exudates from *C. taxifolia* can stimulate bacterial fermentative processes in belowground bacterial communities that in turn change sediment chemistry to favour sulphate reducing microbes (Chisholm and Moulin 2003) and other heterotrophic bacterial groups involved in nutrient uptake (Rizzo et al., 2017). Moreover, there is a significant negative correlation between *C. taxifolia* biomass and sediment boundary-layer dissolved oxygen, sediment redox and the abundance of infaunal organisms (Gribben et al. 2013). This suggests that the biomass of native and/or invasive macrophytes may have important implications for associated sediment microbial communities, sediment functioning and the outcome of interactions between native and invasive macrophytes along biomass gradients.

In harbours and estuaries around Sydney and New South Wales (Australia) more generally, disturbances from boat propellers and anchors, and chains from swing moorings create spatial heterogeneity in biomass in seagrass beds (Short and Wyllie-Echeverria 1996; Glasby and West 2018). In this study, we first conducted field surveys to determine the relationships between *Z. muelleri* and *C. taxifolia* biomass, sediment characteristics and bacterial communities in rhizospheres and bulk sediments. We predicted that the co-occurring native *Z. muelleri* and invasive *C. taxifolia* would differ in their associated bacterial communities and predicted functional capacity, with *C. taxifolia* supporting specific microbial taxa and corresponding metabolic capacities (e.g. sulphate reducers and obligate anaerobes) compared to seagrasses. Similar studies have used spatial variation as a proxy of disturbances in seagrass meadows (Bulleri et al. 2020) but also in other coastal systems (e.g. Douglas et al. 2018; Gladstone-Gallagher et al. 2019; Kraan et al. 2020). Secondly, the influence of each macrophyte on predicted microbial communities would be stronger in the rhizosphere than the bulk sediments, but overall, both the abundance taxa and functional profiles would be determined by the biomass of each macrophyte. Leveraging the spatial variation in the biomass of *Z. muelleri* across a seagrass meadow, we also conducted a field experiment testing the effects of seagrass biomass and *C. taxifolia* fragment propagule pressure on *C. taxifolia* success. Here, we predicted that invasion success would increase as seagrass biomass decreased and would be dependent on propagule pressure.

Materials and methods

Study site and species

This study was performed at Careel Bay, Pittwater in New South Wales, Australia (33.61372222, 151.32461111), a sheltered estuarine system located approximately 25 km north of central Sydney (Suppl. Figure 1). Pittwater estuary has an average depth of less than 5 m and a mean tidal range of 1.8 m making it suitable for snorkel-based manipulation. Pittwater receives little freshwater input due to its location near the estuary mouth, resulting in a salinity range of 30–35 ppt (Bell et al. 1988). *C. taxifolia* was

first described in the bay in December 2000 (Creese et al. 2004), with evidence of its high invasive success through asexual propagation via fragmentation, driven by both natural and human-derived factors (West et al. 2007, 2009). At the time of this study, the benthic community was characterised by areas of uninvaded *Z. muelleri*, uncolonized patches of sand and zones of intermingling *Z. muelleri* and *C. taxifolia*. The availability of uninvaded seagrass and the natural spatial variability in *Z. muelleri* biomass/cover (used as a proxy for disturbance) make Careel Bay an ideal model system for exploring *in-situ* mechanisms behind the establishment of *C. taxifolia*.

Field survey

Previous studies, including those at our study site (e.g., Bulleri et al. 2020), indicate that below-ground processes are stronger mediators of interactions between *Caulerpa* spp. and native seagrasses than above-ground processes (Gribben et al. 2018). To determine the influence of *Z. muelleri* and *C. taxifolia* on the below-ground sediment characteristics and bacterial communities, two distinct sediment fractions were analysed in seagrass beds (i.e., bulk sediments and rhizosphere). Sampling of both sediment fractions was done in Careel Bay on 4th September 2017 (Suppl. Figure 1). Samples were collected in a gradient design determined by percent covers of *Z. muelleri* and *C. taxifolia*. Seagrass cover in plots ranged from 0 to 90%, and *C. taxifolia* cover in plots ranged from 0 to 80%. To compare the potential interactive effects of *Z. muelleri* and *C. taxifolia* on sediment characteristics and bacterial communities, we sampled plots that had 0–90% cover of seagrass without *C. taxifolia* present ($n=13$ plots). We then sampled across the same seagrass cover range but within plots with differing covers of *C. taxifolia* beneath the canopy ($n=15$ plots). Using this design, we investigated potential changes in sediment characteristics and below-ground bacterial communities in seagrass at differing biomass of *C. taxifolia* and across a biomass gradient of *Z. muelleri*.

In each plot, 50 ml of bulk sediment was collected at a depth of 5 cm, as *C. taxifolia* rhizoids and *Z. muelleri* roots can penetrate sediments to this depth (Gribben et al. 2017, 2018). Sediment samples used for grain size and organic content and were placed on

ice immediately after collection. Additionally, 2 ml of the surficial sediment was collected for DNA extraction and placed in a dry-shipper with liquid nitrogen *in situ*. Grain size analyses were performed on wet sediments using a Malvern laser particle size analyser (Mastersizer 2000, USA) and the percentage of silt content ($\% < 63 \mu\text{m}$) was determined using the function *granstat* in the *G2Sd* R package (Fournier et al. 2014). Organic content was measured by the loss on ignition (LOI) method, following a modified protocol used in previous studies (Gonzalez et al. 2021; Walker et al. 2024). Briefly, samples were initially dried at 105 °C for 12 h to remove moisture and then organic matter burned in the muffle furnace at 500 °C for 12 h (Wang et al. 2011). The percentages of LOI were then multiplied by 10 ($1\% = 10 \text{ mg/g}$) followed by the calculation of % organic carbon $= 0.43 * \% \text{ LOI} - 0.33$ by Fourqurean et al. (2012) to estimate the total organic carbon (TOC) in $\text{mg} \cdot \text{g}^{-1}$ (Hyland et al. 2005).

Rhizosphere samples from *C. taxifolia* and *Z. muelleri* individuals within each plot were collected by carefully pulling the plants from the sediment and gently shaking them in water *in situ* to remove loosely attached sediment. The rhizomes/roots were then placed into sterile cryogenic tubes and subsequently stored *in situ* in a dry-shipper filled with liquid nitrogen. All vegetation was then collected from each plot with rhizospheres kept intact. Seagrass was separated from *C. taxifolia* and then washed carefully to remove all sediments. Roots and shoots were separated for dry weight analysis. Samples were weighed before and after drying in a drying oven at 50 °C for 24 h for an estimate of dry weight biomass of each compartment.

Molecular analysis and bioinformatics

Total DNA was directly extracted from 0.25 g of sediment (bulk sediment samples) or obtained from sediment attached to macrophyte roots/rhizomes (Rhizosphere). Rhizomes were vortexed to obtain as much sediment as possible for DNA extraction. For both sediment fractions, total DNA was extracted by using the PowerSoil DNA extraction kit (QIAGEN, Carlsbad, CA, USA) according to the manufacturer's protocol. DNA concentrations were quantified using a NanoDrop ND-2000 (NanoDrop Technologies, Wilmington, DE, USA). Sequencing was done on a

MiSeq Sequencing Platform (Illumina) following the manufacturer's guidelines. Briefly, amplicons targeting the V1-V3 hypervariable region of the bacterial 16S rRNA gene (27F, AGAGTTTGATCMTGGCTCAG, and 519R, GWATTACCGCGGCKGCTG; Lane 1991) were obtained (PCR, with conditions set to a pre-heating step of 95 °C for 3 min, 35 cycles at 95 °C for 15 s, 55 °C for 1 min and 73 °C for 30 s) and consequently sequenced by Ramaciotti Centre for Genomics (UNSW, Australia).

The quality of demultiplexed paired-end raw sequences was visually assessed through quality profiles (package *dada2*, Callahan et al. 2016) and filtering and trimming parameters set based on median Q score > 20 and maximum expected errors (maxEE=2) across samples. Trimmed paired sequences were merged and an additional quality step was performed to remove dimer sequences. Unique sequences were dereplicated, denoised (*-unoise3* command, UNOISE algorithm) and potential chimeric sequences removed (*-uchime2_ref* command with chimera detection based on user-defined database including both GTDB bac120 and GTDB arq53 releases; Parks et al. 2021). A zero-radius Operational Taxonomic Units (zOTUs) table was constructed, and taxonomic classification determined. Taxonomic classification was initially done with SILVA v.138.1 to identify chloroplast and mitochondrial sequences. A second taxonomic classification was also performed with GTDB v214.1 to obtain higher taxonomic resolution. All steps of the bioinformatic pipeline after sequence filtering and trimming were conducted with the software package USEARCH (v.11.0.667, Edgar and Flyvbjerg 2015). All raw paired-end sequences and associated metadata used in this study, are publicly available at the NCBI SRA repository under the BioProject ID PRJNA1128002.

Sequences assigned to chloroplast, mitochondrial plasmid DNA and non-bacterial taxa (i.e., Eukaryota, Archaea or unidentified Kingdom) were removed from the final zOTU table. Contaminating sequences were also removed based on control samples (i.e. prevalence method, R package *decontam*, Davis et al. 2018). At this stage, the zOTU table was divided into two sub-datasets that corresponded to sampled compartments: (1) bulk sediments and (2) macrophyte rhizospheres. For each dataset, singleton, and low abundance sequences (< 0.001% relative abundance across all samples) were also filtered. To address

inherent heterogeneity of library sizes and sparsity of the data, a scaling normalization using a median of ratios method was used independently in both datasets (package *DESeq2*, Love et al. 2014).

Predicted functional profiles were also obtained for the rhizosphere dataset to determine potential differences in the bacterial metabolic capacity between *Z. muelleri* and *C. taxifolia* rhizospheres, considering varying levels of biomass for both macrophytes. A whole-genome reconstruction was done using the available 16S rRNA gene amplicons, using the software PICRUST2 v.2.4.0 (ran through Python v.3.9; Douglas et al. 2020). All zOTU unique sequences were aligned to a reference phylogenetic tree (HMMER, Wheeler and Eddy 2013; EPA-NG, Barbera et al. 2019; and GAPP, Czech et al. 2020) to predict the best reconstructed genome and determine the overall number of copies for each gene family of each zOTU (Louca and Doebeli 2018). The enzyme commission numbering system (EC number, IUBMB) was used to predict gene families that were consequently grouped to inferred metabolic pathways (MetaCyc pathways, MinPath, Ye and Doak 2009). Predicted gene copies for each gene family were normalized to account for varying abundance between zOTU across all samples. Nearest-sequenced taxon indices (NSTI) were calculated for each zOTU to evaluate the quality of the reconstructed genome (NSTI < 2: good quality of predictions according to Langille et al. (2013)).

Statistical analysis

Linear relationships between sediment characteristics (i.e., organic carbon and proportion of fine sediments) and macrophyte cover and biomass across experimental plots were evaluated through a Pearson correlation matrix (sig. $p < 0.05$, R package *corrplot*, Wei et al. 2013). Alpha diversity indices (Richness, Shannon–Wiener diversity and Pielou Evenness index) were calculated for both sediment and rhizosphere datasets to determine the influence of sediment characteristics and macrophyte biomass gradients on these metrics (R package *phyloseq*, McMurdie and Holmes 2013). A Pearson linear regression matrix was constructed to compare alpha diversity metrics with sediment characteristics and macrophyte cover and biomass. In contrast, for rhizosphere data, general linear models for each alpha diversity metric were

constructed with ‘rhizosphere type’ as a fixed factor (2 levels: *C. taxifolia* and *Z. muelleri* rhizosphere), seagrass and *C. taxifolia* biomass as covariates and including all interaction terms. Model inferences were evaluated through an ANCOVA (type III, R package car, Fox and Weisberg 2018) and the model was validated for linearity, normality, and homogeneity of variance (R package performance, Lüdtke et al. 2021).

A multivariate analysis was conducted for bulk sediments and rhizosphere datasets to determine the influence of *Z. muelleri* and *C. taxifolia* biomass on bacterial community structure and composition. A Permutational Multivariate Analysis of Variance was done for each dataset (PERMANOVA, PERMANOVA + v.1.0.5 and Primer-e v.6.1.15; Clarke and Gorley 2006; Anderson et al. 2008), based on a squared-root transformed Bray–Curtis similarity matrix (i.e., zOTU relative abundance) and a Jaccard resemblance matrix (i.e., zOTU presence/absence). For bulk sediments, *Z. muelleri* and *C. taxifolia* biomass and their interaction, were included as the only covariates. In comparison, for the rhizosphere dataset, two separate analyses were conducted to (1) determine differences in bacterial community structure and composition between rhizospheres from *Z. muelleri* and *C. taxifolia* (fixed factor: ‘rhizosphere type’ with two levels, PERMANOVA as above) and (2) including *Z. muelleri* and *C. taxifolia* biomass as covariates potentially affecting bacterial community structure and composition in the rhizosphere (dbRDA, see description below). For the former analysis, experimental plot was added as a random factor to account for plots with the presence of both macrophytes. A Permutational Analysis of Dispersion (PERMDISP, PERMANOVA + v.1.0.5) was also included in this analysis to determine differences in structural and compositional dispersion from the centroids between ‘rhizosphere type’. To visualize the multivariate results, a principal coordinate analysis was conducted and, when evaluating the effects of covariates (i.e., macrophyte biomass), a distance-based redundancy analysis and a permutation test to determine significant marginal effects of covariates were conducted (dbRDA, R package vegan, Oksanen et al. 2019). Ellipses representing the standard error of the weighted average distance from the centroid were also included for the factor, ‘rhizosphere type’, in these ordinations.

To further explore the differences between rhizosphere types and the influence of *Z. muelleri* and *C. taxifolia* biomass, a differential abundance analysis was conducted to identify specific bacterial zOTUs. Briefly, this analysis consisted of fitting the zOTU abundance data into a generalised linear model under a negative binomial distribution that includes the factors and covariates as above for each dataset (R package mvabund, Wang et al. 2012). Model inferences for each factor and covariates were evaluated through an analysis of deviance and, to detect significant changes in the abundance of specific zOTUs, univariate tests per each zOTU were obtained and corrected for multiple testing through a stepdown resampling method (Wang et al. 2012). A selection of the most abundant (>0.2%) and prevalent zOTUs (present in >80% of all samples) that differed across evaluated factors was conducted. General linear models as above were conducted for these selected taxa to determine the effects of factors (i.e., rhizosphere type) and covariates (i.e., macrophyte biomass) on their total abundance.

A similar multivariate analysis approach as described above (i.e., PERMANOVA, PERMDISP and dbRDA) was performed to determine differences in predicted functional profiles between ‘rhizosphere type’ and biomass of *Z. muelleri* and *C. taxifolia*. This analysis used a similar approach for constrained ordinations including a dbRDA, covariate fitting (*envfit*) and smooth response surfaces (*ordisurf*). Additionally, filtering of pathways under the MetaCyc classes anaerobic respiration, nitrogen and of sulphur compound metabolisms, and polysaccharide degradation, was conducted and analysed further. This included univariate linear models including ‘rhizosphere type’ as main factor, macrophyte biomass as covariates and all interactions therein. All statistical analysis were conducted in R v.4.3.1 and visualizations created in *ggplot* v3.4.2 (Wickham et al. 2016).

Manipulative field experiment

To determine the effects of *Z. muelleri* biomass and *C. taxifolia* propagule pressure on the establishment and growth of *C. taxifolia* fragments, a manipulative experiment was conducted in a 20×20 m uninvaded section of the seagrass bed (although *C. taxifolia* does occur in other sections of the bed) over a 5-week period from 28th March to 1st May 2018. The experiment duration was based on similar studies showing

that 5 weeks is sufficient to determine treatment effects (Uyà et al. 2018; Gribben et al. 2018; Bulleri et al. 2020). Sixty-two plots (0.5×0.5 m) were set up across the full range of naturally occurring seagrass cover (ranging from 0 to 92% cover) and each plot was thoroughly examined to ensure complete absence of *C. taxifolia* prior to the experiment. All plots were positioned at least 3 m away from large unvegetated areas to avoid edge effects (Murcia 1995; Statton et al. 2015). Similarly, neighbouring plots were positioned at least 2 m apart to ensure no interaction between treatments. Plots of differing cover were spatially intermingled to minimise any other confounding gradients in environmental conditions that may have influenced the results.

To create the propagule pressure treatments, *C. taxifolia* fragments were sourced from invaded areas of the seabed within Careel Bay (~100 m from the experimental area) and cut to 10 cm long fragments, each with three approximately equal sized fronds and four rhizoid clusters (Bulleri et al. 2020). On the same day, fragments were transplanted into the centre of the plots and secured with two U-shaped metal pins per fragment (Ceccherelli et al. 2000; Piazzini et al. 2005; Gribben et al. 2018). Half of the plots received eight (high propagule pressure) fragments, and the remainder received four fragments (low propagule pressure; after Uyà et al. 2018; Bulleri et al. 2020). Propagule pressure treatments were established so that both treatments covered the spectrum of seagrass cover at the site. In addition, this experimental setup minimized artefacts of stress from transplantation and manipulation while ensuring the survival and growth of fragments throughout the experiment (e.g. as utilised in Wright and Davis 2006; Bulleri et al. 2020). After five weeks, all *C. taxifolia* fragments in each plot were collected, placed in labelled plastic bags and stored (at -20 °C) before analysis. All pins were still in place at the end of the experiment although some fragments were no longer present. Following the removal of *C. taxifolia* fragments, we removed *Z. muelleri* from a 25×25 cm core placed at the centre of each plot.

In the laboratory, the seagrass samples were divided into above- (shoots and blades) and below-ground (roots) biomass, allowing the characterisation

of the relative contributions of each to overall seagrass biomass. Subsamples were dried (for 48 h at 60 °C) and weighed. *C. taxifolia* fragments from each plot were photographed (Suppl. Figure 2B) and examined for changes in morphology in response to seagrass biomass and propagule pressures. Fragment photographs were analysed with *ImageJ*™ (v1.51j8, Schneider et al. 2012). For each plot, we determined the number of fronds, length of each frond, rhizoid number and stolon length. We then determined total fragment biomass in each plot by drying (at 60 °C for 48 h) and weighing all the *C. taxifolia* collected within each plot. We could not analyse individual fragments, or frond and rhizoid measures per fragment as many fragments broke-up once placed in bags.

Statistical analysis

Linear models were used to determine the effects of total seagrass biomass, together with propagule pressure, on *C. taxifolia* establishment. Each model included only *Z. muelleri* total biomass as a continuous covariate (i.e. total, root and shoot biomass), propagule pressure as a fixed factor (two levels: high and low) and interactions therein, with total *C. taxifolia* biomass per plot as the response variable. Total biomass was selected as the continuous covariate as initial models did not find any difference in the results obtained by analysing solely above (shoots) or on belowground biomass (roots) separately. Additionally, above- and below-ground seagrass biomass was correlated directly with total biomass, and this latter value was considered to be a better standardized and less variable measure of seagrass biomass. The same approach was used to determine the effect of *Z. muelleri* biomass and propagule pressure on other *C. taxifolia* morphometrics (i.e., frond number, frond length, rhizoid number and stolon length). All model inferences were evaluated through an ANCOVA (type III, R package *car*, Fox and Weisberg 2018) and assumptions were validated (R package *performance*, Lüdtke et al. 2021). If model assumptions were not met, data was transformed. All statistical analyses were performed using R version 3.4.3.

Results

Sediment and microbial profiles across biomass gradients of *Z. muelleri* and *C. taxifolia*

A distinct seagrass biomass gradient (ranging 0–27 g dw) was effectively sampled in the surveyed area and was linearly correlated with the observed seagrass cover (Suppl. Table 1, $R=0.84$, $p<0.05$). The largest proportion of seagrass biomass occurred below ground (i.e., average root and rhizosphere biomass $5.32 \text{ g dw} \pm 0.88 \text{ SE}$) compared to above ground tissues (i.e., leaves, $1.53 \text{ g dw} \pm 0.26$). *C. taxifolia* biomass in the same plots (biomass range 0–4 g dw/plot and average $1.21 \text{ g dw} \pm 0.25 \text{ SE}$) was comparatively lower than seagrass (biomass range 0–27 g dw/plot and average $6.86 \text{ g dw} \pm 1.12 \text{ SE}$) and was also strongly correlated to their observed cover (Suppl. Table 1, $R=0.89$, $p<0.05$). There was no relationship between both macrophyte biomass gradients and organic carbon or proportion of fine sediments (Suppl. Table 1).

A total of 11,267 bacterial zOTUs occurred in samples taken from bulk sediments. The predominant bacterial classes found across all experimental plots (relative abundance $>5\%$) included Gammaproteobacteria (28%, with 106 genera identified), Acidimicrobiia (8%, 9 genera), Bacteroidia (7%, 72 genera), Alphaproteobacteria (5%, 133 genera), Desulfobacteria (5%, 13 genera) and Planctomycetes (5%, 14 genera). Taxonomic assignment of approximately 29% of zOTUs sequences could not be resolved to class level (i.e., unidentified bacterial zOTUs). An overall summary of the most dominant bacterial classes across the bulk sediment samples is in Suppl. Figure 2. All bacterial alpha diversity metrics (diversity and evenness, Suppl. Table 1), and community structure (Bray–Curtis) and composition (Jaccard index, Suppl. Table 2), estimated from bulk sediment samples were unrelated to gradients of seagrass or *C. taxifolia* biomass except for bacterial richness which decreased with increasing seagrass biomass although the relationship was weak (Suppl. Table 1, $R=-0.39$, $p=0.04$). There were no significant relationships between bulk sediment bacterial alpha diversity metrics and other sediment properties (i.e., organic carbon or fine sediments proportion, Suppl. Table 1). Seagrass and *C. taxifolia* biomass were unrelated to dissimilarities

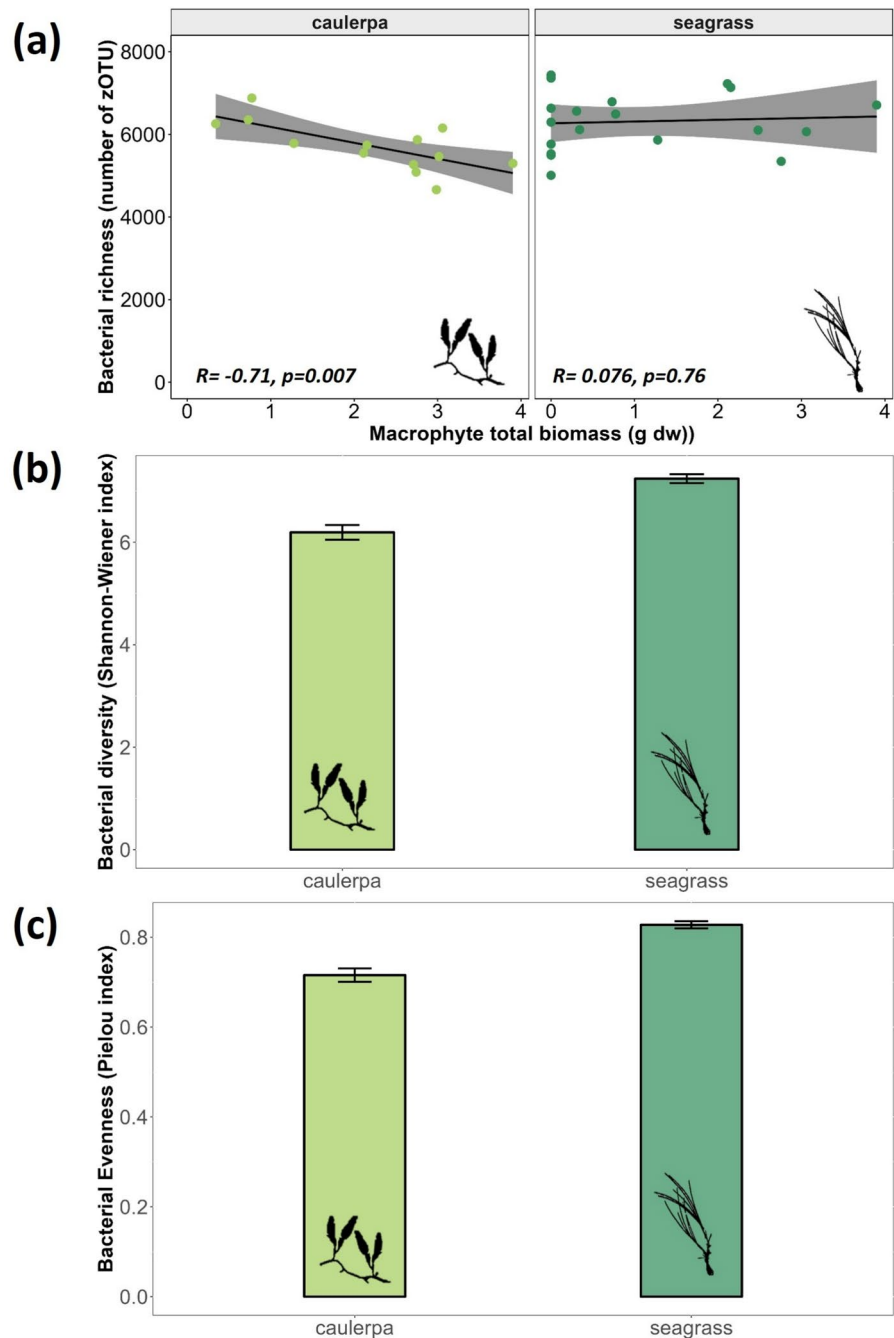
in bulk sediment bacterial community structure or composition (Suppl. Table 2, PERMANOVA).

A total of 390 metabolic pathways were inferred to construct an adequate prediction of the functional profile in bulk sediments (weighted NSTI = 0.46 ± 0.01 , Langille et al. 2013). Within these, most pathways ($>1\%$ relative abundance across samples) were associated with aspects of cellular maintenance including biosynthesis (63.7%) and generation of precursor metabolites and energy (11.93%), as well as compound degradation, utilization, or assimilation (8.84%). From the latter category, the main predicted metabolic pathways with high relative abundance across samples ($>0.5\%$) included CO_2 fixation (2.05%), purine nucleotide degradation (1.12%), sugar degradation (0.93%), polysaccharide degradation (0.65%), fatty acid/lipid degradation (0.62%), proteinogenic amino acid degradation (0.55%) and sulphur compound metabolism (0.60%).

For microbial samples taken directly from seagrass and *C. taxifolia* rhizospheres, a total of 10,124 bacterial zOTUs were detected which corresponded to 66 classes across all samples (Suppl. Figure 3). Rhizospheres of *C. taxifolia* were dominated (relative abundance $>2\%$) by the classes Desulfobulbia ($35\% \pm 2.04\% \text{ SE}$), Desulfobacteria ($20\% \pm 3.37\% \text{ SE}$), Gammaproteobacteria ($10\% \pm 0.75\% \text{ SE}$), Bacteroidia ($4\% \pm 0.41\% \text{ SE}$), Alphaproteobacteria ($4\% \pm 0.43\% \text{ SE}$), Desulfuromonadia ($2\% \pm 0.47\% \text{ SE}$) and Acidimicrobiia ($2\% \pm 0.21\% \text{ SE}$). *Z. muelleri* rhizospheres were dominated by the classes Gammaproteobacteria ($20\% \pm 1.23\% \text{ SE}$), Desulfobacteria ($11\% \pm 0.67\% \text{ SE}$), Bacteroidia ($9\% \pm 0.47\% \text{ SE}$), Alphaproteobacteria ($7\% \pm 0.77\% \text{ SE}$), Desulfobulbia ($6\% \pm 0.63\% \text{ SE}$), Campylobacteria ($3\% \pm 0.25\% \text{ SE}$), Acidimicrobiia ($3\% \pm 0.29\% \text{ SE}$) and Clostridia ($2\% \pm 0.25\% \text{ SE}$). Taxonomic assignment could not resolve the classification to a class level of 16% zOTUs in rhizospheres of *C. taxifolia* and 32% zOTUs in rhizospheres of *Z. muelleri*.

Bacterial alpha diversity and evenness was higher on seagrass rhizospheres compared to *C. taxifolia* rhizospheres (Fig. 1b,c and Suppl. Table 3; Diversity: increase of 14.5%, $F_{1,24}=41.48$, $p<0.001$; and Evenness: increase of 13.5%, $F_{1,24}=30.65$, $p<0.001$). Bacterial richness was also higher in seagrass compared to *C. taxifolia* rhizospheres, although

Fig. 1 Bacterial alpha diversity indices (a) richness (number of zOTUs), (b) diversity (Shannon–Wiener index) and (c) evenness (Pielou index) on the rhizospheres of *Caulerpa taxifolia* (n = 13) and the seagrass *Zostera muellerii* (n = 19). For (a), a linear regression was separately fitted between bacterial richness and the biomass (g dw) of each macrophyte. (b) and (c) describe the mean $\pm$ SE of each alpha diversity index between the two rhizosphere types. Significant differences in (b) and (c) are shown with an asterisk (*)



richness of the seagrass rhizosphere decreased at higher *C. taxifolia* biomass (Fig. 1a, Suppl. Table 3, rhizosphere type $\times$ *C. taxifolia* biomass interaction; $F_{1,24} = 41.48, p = 0.017$). Structural and compositional dissimilarities of bacterial communities differed between rhizospheres of seagrass and *C. taxifolia* (Fig. 2, Suppl. Table 4A, PERMANOVA,

Bray–Curtis: pseudo- $F_{1,31} = 11.38, p < 0.001$; Jaccard: pseudo- $F_{1,31} = 7.96, p < 0.001$). Additionally, higher structural variability occurred on seagrass compared to *C. taxifolia* rhizospheres (Fig. 2a, PERMDISP, Bray–Curtis, $F_{1,31} = 8.01, p = 0.01$). When analysing the samples taken only from bacterial communities on seagrass rhizospheres, structure and composition

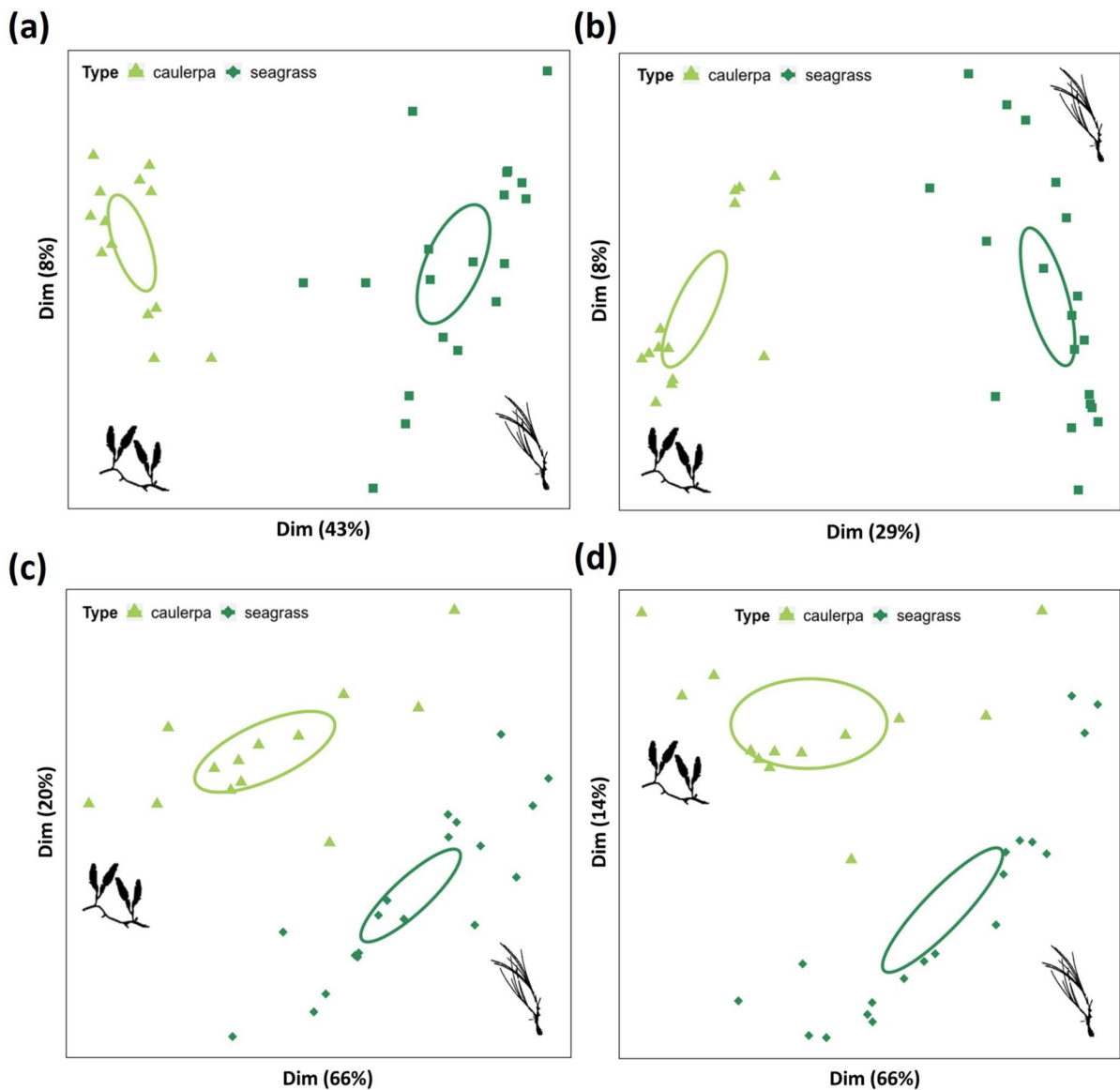


Fig. 2 Principal coordinate analysis ordinations showing the differences in structure (Bray–Curtis, a,c) and composition (Jaccard index, b,d) of bacterial communities (a,b) and associated predicted functional profiles (c,d) between samples taken

from *Z. muelleri* and *C. taxifolia* rhizospheres. Ellipses represent the standard error of the points from the centroid of each group. Functional profiles were predicted from reconstructed genomes (*Picrust2*)

was unrelated to either *C. taxifolia* or seagrass biomass (Suppl. Table 4B, marginal effects dbRDA).

Differential abundance analyses showed 1309 zOTUs were related to rhizosphere type and macrophyte biomass, although only 7 zOTUs were highly abundant (relative abundance > 0.2%) and prevalent (frequency > 80%) across all samples (Suppl. Table 6). Individual univariate tests on these abundant zOTUs,

showed that overall, 4 genera differed between the macrophyte rhizospheres and changed with macrophyte biomass. The genera *Desulfobacula* (zOTU 70, with average abundance of 367.42 ± 117.4 SE reads) and *Thiodiazotropha* (zOTU 1816 with of 616 ± 116 reads, zOTU7614 and zOTU 319 with 516 ± 90.7 SE) had higher abundances in seagrass compared to *C. taxifolia* rhizospheres (< 2.3 reads across all genera,

Suppl. Table 6). *Thiodiazotropha* (a sulphide-oxidizing chemoautotrophic Gammaproteobacteria; Martin et al. 2020a, b) abundance on seagrass rhizospheres also appeared to decrease at increasing levels of seagrass biomass when *C. taxifolia* presented biomass of 3 g dw (Suppl. Table 6). In comparison, the genus *Desulfatitalea* (zOTU4 and zOTU12) had higher abundance in *C. taxifolia* compared to seagrass rhizospheres (abundance ~98% higher), although abundances were dependent on the biomass of both macrophytes (Suppl. Table 6). On seagrass rhizospheres, the abundance of *Desulfatitalea* decreased with increasing biomass of *C. taxifolia* and decreasing biomass of seagrass. On *C. taxifolia* rhizospheres, the abundance of *Desulfatitalea* decreased with decreasing *C. taxifolia* biomass and was unrelated to seagrass biomass present.

Predictability for genome reconstruction used to infer bacterial functional profiles was adequate for the rhizosphere dataset (i.e., based on 400 inferred pathways, weighted NSTI = 0.03 ± 0.01 , Langille et al. 2013). Composition of these predicted functional metabolic profiles differed between rhizosphere type (Fig. 2c,d, Suppl. Table 5A, Bray Curtis: PERMANOVA, pseudo- $F_{1,31} = 16.32$, $p < 0.001$; Jaccard: PERMANOVA, pseudo- $F_{1,3} = 14.28$, $p < 0.001$) and with no differences in compositional intragroup variability (Fig. 2c,d, PERMDISP, Bray–Curtis: $F_{1,31} = 0.68$, $p = 0.8043$; Jaccard: $F_{1,31} = 0.68$, $p = 0.8043$). Predicted functional profiles on samples exclusively from *Z. muelleri* rhizospheres were unrelated to seagrass or *C. taxifolia* biomass (Suppl. Table 5B, marginal effects).

Four metabolic pathway categories based on MetCyc data were selected from the complete profiles to be explored further: (i) anaerobic respiration, (ii) nitrogen compound metabolism, (iii) sulphur compound metabolism and (iv) polysaccharide degradation. Across all these categories, only 8 metabolic pathways differed between rhizosphere types ($F_{1,27} > 5.8$ and $p < 0.05$ across all 8 pathways) and were unrelated to the biomass of either macrophyte. Higher abundance of bacterial gene copies occurred on seagrass compared to *C. taxifolia* rhizospheres including for starch degradation (i.e. 25.73% higher, $F_{1,27} = 7.39$, $p = 0.011$), nitrogen reduction (i.e. denitrification 84.63% higher, denitrification: $F_{1,27} = 79.14$, $p = 0.023$; and assimilatory 64.96% higher: $F_{1,27} = 16.06$,

$p < 0.001$), sulphate reduction (28.93% higher, $F_{1,27} = 23.81$, $p < 0.001$) and sulphate assimilation (15.96% higher, $F_{1,27} = 6.48$, $p = -0.017$). By contrast, bacterial gene copies were higher in *C. taxifolia* rhizospheres compared to seagrass rhizospheres in pathways such as homolactic fermentation (32.25% higher, $F_{1,27} = 5.80$, $p = 0.023$), urea cycling (53.28% higher, $F_{1,27} = 79.37$, $p < 0.001$) and sulphide oxidation (61.67% higher, $F_{1,27} = 31.34$, $p < 0.001$).

Effect of seagrass biomass and propagule pressure on *C. taxifolia* establishment success

The total dry biomass of seagrass and propagule pressure had interactive effects on total *C. taxifolia* dry biomass in plots at the end of the experiment (Fig. 3a, $F = 6.4$, $p = 0.015$). *C. taxifolia* dry biomass decreased with increasing seagrass biomass at high levels of propagule pressure (i.e., 8 fragments/plot). At lower levels of propagule pressure (i.e., 4 fragments/plot) *C. taxifolia* biomass also decreased with increasing seagrass biomass, but at a lower magnitude of change. Similar results occurred for most *C. taxifolia* morphometric measurements including stolon length (Fig. 3b, $F = 6.16$, $p = 0.016$) and number of rhizoids (Fig. 3d, $F = 6.77$, $p = 0.012$). Number of *C. taxifolia* fronds also decreased in plots with higher biomass of *Z. muelleri* but this was independent of the propagule pressure treatment (Fig. 3c, $F = 14.25$, $p < 0.001$). *C. taxifolia* frond length did not vary with propagule pressure or seagrass biomass (see full model results in Table 1).

Discussion

Although several theories underpinning invasion success have received empirical support, the interaction between different mechanisms controlling the establishment of invasive species is less well-explored. Here we show through a field survey, that bacterial taxonomic structure and potential metabolism differed between the native seagrass (*Zostera muelleri*) and the invasive alga *Caulerpa taxifolia*. Our results also indicate that invasion success appeared mostly unrelated to measured sediment characteristics. However, we observed shifts in the abundance of some key rhizosphere-associated

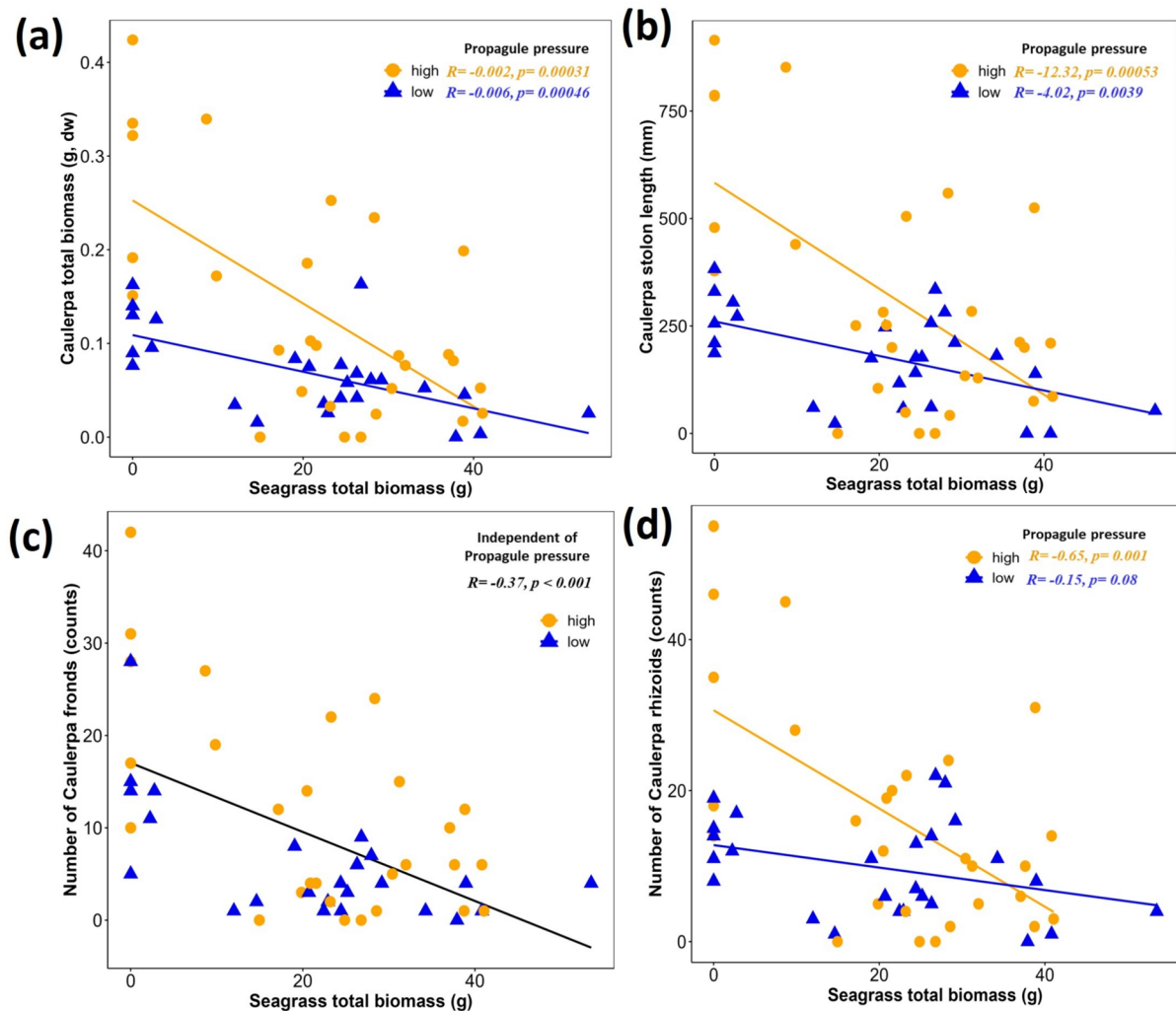


Fig. 3 Linear relationships between (a) total *C. taxifolia* biomass and three morphometric measurements (b): stolon length, (c): number of fronds and (d): number of rhizoids; and the total of dry *Z. muelleri* biomass ($n=62$). In (c), the black line

represents pooled data from propagule pressure treatments due to the absence of a significant interaction. Significant correlations are indicated by regression lines ($p < 0.05$)

bacteria (e.g., *Thiodiazotropha* and *Desulfatitalea*) across biomass gradients of both macrophytes suggesting invasion success may be mediated by interactions between root-associated microbes that intensify with increasing seagrass and *C. taxifolia* biomass. In addition, the manipulative experiment showed that native plant biomass and propagule pressure have interactive effects on the final biomass of *C. taxifolia*. As expected, final biomass of *C. taxifolia* decreased with increasing native seagrass biomass, however, these negative effects were stronger at higher propagule pressure.

The effect of *Z. muelleri* biomass on *C. taxifolia* invasion success

Increasing *Z. muelleri* biomass was significantly correlated with a reduction in *C. taxifolia* biomass in the experimental manipulation (total biomass, stolon, fronds and rhizoids). These results show that, like other plant communities (e.g., Davis 2000; Holle and Simberloff 2005), an increase in biomass or cover of the native seagrass *Z. muelleri* can enhance resistance to biological invasions of non-native macrophytes. This highlights the strong resistance of seagrass beds

Table 1 Summary of ANCOVA evaluating the effect of *Z. muelleri* total biomass (covariate), two propagule pressure treatments (fixed factor, low and high) and interactions therein,on *C. taxifolia* biomass and four morphometric measurements (stolon length, number of fronds, frond length and number of rhizoids)

Caulerpa measurement	Source	Sums of Squares	df	F value	P value
Total biomass (g, dw)	Propagule pressure (PP)	0.083	1	16.29	0.0002
	Seagrass total biomass	0.15	1	29.31	< 0.0001
	PP x Seagrass total biomass	0.033	1	6.4	0.015
	Residuals	0.255	50	NA	
Stolon Length (mm)	Propagule pressure (PP)	418,581.977	1	14.36	0.0004
	Seagrass total biomass	751,386.916	1	25.78	< 0.0001
	PP x Seagrass total biomass	179,519.891	1	6.16	0.016
	Residuals	1,457,214.539	50	NA	
sqrt (Number of fronds)	Propagule pressure (PP)	4.87	1	2.92	0.094
	Seagrass total biomass	23.804	1	14.25	0.0004
	PP x Seagrass total biomass	0.754	1	0.45	0.505
	Residuals	83.537	50	NA	
log (Frond length + 0.001)	Propagule pressure (PP)	1.202	1	0.11	0.74
	Seagrass total biomass	18.421	1	1.7	0.2
	PP x Seagrass total biomass	1.268	1	0.12	0.73
	Residuals	540.536	50	NA	
Number of rhizoids	Propagule pressure (PP)	1,282.716	1	13.27	0.001
	Seagrass total biomass	2,093.977	1	21.66	< 0.0001
	PP x Seagrass total biomass	654.688	1	6.77	0.012
	Residuals	4,833.464	50	NA	

Significant effects are shown in bold and highlighted in grey

to the establishment of *C. taxifolia* regardless of propagule pressure, with *C. taxifolia* largely restricted to establishing in highly disturbed plots. Inter-specific competition for light and space (Bernardeau-Esteller et al. 2015) and production of phenolics (Firth et al. 2024) are possible explanations for the effects of seagrass biomass on *C. taxifolia* fragments. Effects of competition were observed through the effects on *C. taxifolia* morphological traits (i.e. stolon length, number of fronds, and number of rhizoids) all of which were reduced at higher seagrass biomass, except for frond length. Reductions in morphological traits of fragments could also present an adaptive response to increased competition for light where resource allocation is focused on maintaining frond length rather than frond number (Ceccherelli and Cinelli 1998; Bulleri et al. 2020). Since *Z. muelleri* at the experimental site grows in a relatively shallow subtidal habitat with low turbidity and reaches short lengths (approx. 10 cm), and we have previously demonstrated *C. taxifolia* fragments grow similarly in the presence/absence of a *Z. muelleri* canopy at this location (Bulleri et al. 2020), competition for above-ground resources such as light seems unlikely. It is also possible that other environmental factors varied across the different plots, including unmeasured local sediment characteristics (e.g. nutrients such as N or P, redox potential and sulphur concentrations) or long-term legacy effects of disturbances (Kominoski et al. 2020) which may act as potential confounding factors in this study. However, the macrophyte biomass gradient in the field was composed of multiple interspersed plots that showed very little variability in measured sediment characteristics (i.e. average organic carbon across plots: $0.12 \text{ g TOC/g sediment} \pm 0.019 \text{ SE}$; and % of fine sediments: $3.5 \pm 0.32\% \text{ SE}$). This suggests that the influence of these unmeasured factors is likely limited compared to the evaluated variables.

A second possibility for resource competition between macrophytes is for below-ground nutrients. However, our field survey showed that *C. taxifolia* biomass was not correlated to sediment characteristics such as total organic carbon and the proportion of fine sediments. While unmeasured nutrients such as nitrogen or phosphorous could be limited in this system, nutrients sourced from microbially-mediated processes seem unlikely due to the limited differences found in bulk sediment bacterial communities. Bulk sediment bacterial communities in the field survey

were homogenous across all plots and characterised by zOTUs that are included within common bacterial groups found in marine sediments (e.g., members of the families *UBA5794* and *SZUA-442*, Lopez-Sanchez et al. 2024; or the genera *Tenacibaculum* or *Kriegella*; Habib et al. 2014, Bowman 2006) and include likely drivers of local nutrient cycling (e.g. genus *Thiohalomonas* in denitrification pathways; Sorokin et al. 2007; or *Desulfotalea* in sulphate reduction, Song et al. 2021). Overall, bulk sediment bacterial communities were not strongly influenced by gradients of macrophyte biomass apart from a weak linear trend in bacterial richness in plots with higher seagrass biomass. This suggests that the negative effects of seagrass biomass on *C. taxifolia* invasion success seen in the propagule experiment are likely not driven by belowground microbial communities in bulk sediments. Instead, root-associated microbial communities from both macrophytes may play a more significant role, either by actively altering sediment conditions (e.g. shifts in redox state or sulphide production) or by enhancing interspecific interactions.

Results from the field study reinforce evidence from other studies that both *C. taxifolia* and *Z. muelleri* actively influence microbial communities to create unique below-ground environments that largely differ in their metabolic capacities (Holmer et al. 2009; Aires et al. 2015; Korlevic et al. 2021; Gribben et al. 2017; Aldegue-Riquelme 2022). Through their rhizoids, *Caulerpa* species have been observed to alter the surrounding bacterial communities (Gribben et al. 2017), promoting anaerobic metabolic pathways, such as nitrogen fixation, that enhance sediment N availability even within seagrass meadows (Chisholm and Moulin 2003). Broadly in this study, the *C. taxifolia* rhizosphere favoured sulphate-reducing bacterial taxa from the dominant classes Desulfobulbia, Desulfobacteria and Desulfomonadia, the latter being at higher abundances in this alga compared to seagrasses. Some of these classes have been characterized on other *Caulerpa* species (Aires et al. 2015; Korlevic et al. 2021; Aldegue-Riquelme 2022) and are likely to be an active component that enables *C. taxifolia* to develop in anaerobic conditions. Anoxic sediment microenvironments created by *C. taxifolia* can facilitate fermentative processes of anaerobic bacterial groups (Chisholm and Moulin 2003) and this is clearly observed in the predictive functional profiles obtained in this study (e.g.,

homolactic fermentation and the urea cycle). Small-scale partitioning in *C. taxifolia* rhizosphere possibly also exist, permitting small oxic areas to form (as seen in other macrophytes, Thomas et al. 2014) enabling both sulphate reduction and sulphide oxidation to take place. This adaptation in *C. taxifolia* may also enable other metabolic processes such as nitrogen fixation and denitrification (Eyre et al. 2011) to dynamically occur even under highly anoxic conditions. Comparatively, these bacterial groups and functional capacities are significantly reduced in the rhizosphere of *Z. muelleri*, where other groups and metabolic pathways become dominant, posing a direct challenge for *C. taxifolia* to develop.

The production of oxygen in the seagrass rhizosphere tightly regulates microbial communities and their metabolic pathways (e.g., sulphide oxidation, reduction, nitrification and denitrification, Seymour et al. 2018). This tight relationship between seagrass and belowground microbial communities provides a difficult environment for *C. taxifolia* to establish and thrive (Gribben et al. 2017). This effect has been observed in similar experimental manipulations, where *C. taxifolia* fragment success was substantially reduced when exposed to bulk sediments collected from *Z. muelleri* beds (Gribben et al. 2017). The experimental manipulation in the present study produced similar results, *C. taxifolia* biomass decreasing as *Z. muelleri* biomass increased. However, the field survey identified only two bacterial taxa that changed with increasing seagrass biomass. The bacterial genus *Desulfatitalea* which is known to be a dominant sulphate reducing genus (Korlevic et al. 2021) and present in *Caulerpa* epiphytic communities (Aires et al. 2015), decreased with increasing seagrass biomass. Shifts to a more aerobic micro-environment at higher seagrass biomass possibly affected bacterial members of this genus associated with *C. taxifolia*, while providing better conditions for other groups. This may explain increases in abundance of the diazotroph, *Thiodiazotropha* at higher seagrass biomass. This genus appears almost exclusively in seagrass samples and represents a dominant group, colonizing seagrass root systems worldwide (Martin 2020a). *Thiodiazotropha* plays an important role in facilitating symbiotic nitrogen fixation in conjunction with bioturbating macrofauna (i.e., Lucinids, Lim et al. 2019) and alleviates toxic effects of sulphides (i.e. sulphide oxidation, Martin et al. 2020b; Ling et al. 2021). Greater

bacterial capacity for denitrification and nitrogen reduction in seagrasses supports this shift in sediment microenvironment conditions at higher seagrass biomass and aligns with a higher abundance of these diazotrophic bacteria (McGlathery et al. 1998). The high abundance of other bacterial groups such as the class Campylobacter and the genus *Desulfobacula*, which are involved in nitrogen fixation (de Wit 2008) and sulphide oxidation (Miranda et al. 2022), along with indications of high starch degradation capacity (i.e., predicted aerobic metabolic pathway) further supports that the rhizosphere in *Z. muelleri* favours an aerobic environment. These microbial taxa may provide seagrasses with a competitive advantage and resistance towards invasive species like *C. taxifolia*.

Recent experiments, including those at our study site, showed that *C. taxifolia* fragment success was promoted only when seagrass beds were disturbed, and not when seagrass beds remained intact or when seagrass canopies were removed but sediments were left intact (Gribben et al. 2018; Bulleri et al. 2020). Additionally, experimental removal of rhizosphere microbial communities of *Z. muelleri* suggests these communities might play a minor role in its growth unless they are subject to stressful conditions (e.g., nutrient enrichment, Fuggle et al. 2023). In our field survey, the only dominant bacterial taxon in the *C. taxifolia* microbial community that remained unaffected by increasing seagrass biomass was the epiphytic sulphate-reducing genus *Desulfitalea*. It is likely that at high biomass, *C. taxifolia* creates a resilient anaerobic community where vital sulphur-reducing groups such as *Desulfitalea* can thrive, even within a neighbouring aerobic environment created by seagrass roots (Gribben et al. 2018). Under the evaluated conditions, these bacterial groups from *C. taxifolia* may remain isolated in this anaerobic environment or coexist to some extent with the seagrass microbial community. Since many natural and anthropogenic stressors (e.g., ocean acidification, thermal stress, propeller scarring and wasting disease) result in the loss or degradation of seagrass biomass (Hastings et al. 1995; Short and Wyllie-Echeverria 1996; West et al. 2012; Ravaglioli et al. 2024) and alter their rhizosphere systems (Martins et al. 2020a), seagrass beds that are already disturbed may be disproportionately vulnerable to invasions by *C. taxifolia* compared to healthy beds, as observed elsewhere (Ceccherelli et al. 2014). This emphasises the importance of

maintaining healthy and intact seagrass biomass and rhizosphere sediments to preserve their natural resistance to invasions.

The effect of propagule pressure and seagrass biomass on invasion success

Total *C. taxifolia* biomass recovered from plots with low seagrass biomass was twice as high in the high propagule treatment compared to the low propagule treatment, suggestive of additive effects of propagule supply on invasion success. However, increasing seagrass biomass had stronger negative effects on the high propagule pressure compared to the low propagule pressure treatments, such that both treatments had similar *C. taxifolia* biomass at the end of the experiment. Although weak linear relationships and high variability in fragment biomass responses were detected, these trends remained significant and align with previous studies reporting similar results. For example, Uya et al. (2018) found increasing cover of the native seagrass *Posidonia oceanica* reduced the establishment of *C. cylindracea* and individual fragment biomass was reduced at higher compared to lower propagule pressures. This contrasts with studies demonstrating that high propagule pressure can play an important role in non-native species overcoming increased levels of biotic resistance provided by native species (e.g., Holle and Simberloff 2005; or Clark and Johnson 2009).

One explanation is that at higher seagrass biomass, increasing propagule pressure strengthens intra-specific interactions such as competition for resources among *Caulerpa* spp. fragments. For another invasive seaweed, germlings of the invasive alga, *Sargassum muticum*, were strongly impacted by intra-specific competition for nutrients (Steen 2003). In our field study, it is possible that unmeasured nutrients (e.g., N and P) became increasingly limited as higher carbon sources from *Z. muelleri* became more abundant at higher seagrass biomass. However, it is unlikely that light or nutrients were limiting at the experimental site as explained above. One alternative hypothesis is that under low *C. taxifolia* fragment biomass, natural mutualistic microbes in the sediment may provide indirect feedbacks for *C. taxifolia* (van der Putten et al. 2016) that would either offer protection or cause negative effects such as increased toxicity of bacterial

derived compounds (e.g. increasing sulphate production by bacterial reducers). It is also possible, albeit still speculative that at these biomass levels, *C. taxifolia* may also influence the accumulation of pathogens that could significantly disturb *Z. muelleri* (e.g. in terrestrial plants, Mangla and Callaway, 2008). However, at higher fragment abundances, intra-specific interactions may also shift, leading to increased negative effects. These could be driven by a higher concentration of secondary metabolites produced by the fragments (i.e. toxic or as a defense response as in other exotic species, Callaway and Ridenour 2004), a shift of microbial taxa, from previously beneficial role to a pathogenic identity (e.g., facultative pathogens, Saha and Weinberger 2019), or increased susceptibility to external pathogens (Jiang et al. 2020). Coupled with inter-specific competition for resources, pathogen spillover and a predominantly aerobic sediment environment created by *Z. muelleri*, *C. taxifolia* fragments likely had a very low defense against multiple negative feedbacks. Similarly in terrestrial systems, native-plant sediment microbial communities play a crucial role in the establishment of early successional non-invasive and invasive plant species (van der Putten et al. 1993; Jiang et al. 2020). Thus, by increasing the biomass of the invasive algae *C. taxifolia* in this experiment, propagules may have been subjected to an increasingly negative environment created by both intra- and interspecific interactions, ultimately affecting their performance.

Conclusion

Here we showed that two factors—native species biomass and invasive propagule pressure—had important interactive effects on establishment success of the invasive alga *C. taxifolia*. Increasing native biomass reduced fragment success and increasing invasive propagule pressure could not overcome this effect. In fact, high propagule pressure further decreased invasion success at high native species biomass. This suggests that invasion success may be determined by the interplay between inter- and intra-specific interactions. The absence of differences in bulk sediment physical characteristics and microbes, but changes in some key microbial taxa in the rhizosphere associated with increasing biomass of both species, suggests that

increasing native species biomass may restrict invasion success through more frequent root-microbe interactions. We suggest that investigating the effects of root microbes on invasion success is an important avenue for future research.

Funding Open Access funding enabled and organized by CAUL and its Member Institutions.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Aires T, Moalic Y, Serrao EA, Arnaud-Haond S (2015) Holocene theory supported by cooccurrence networks of species-specific bacterial communities in siphonous algae (*Caulerpa*). *FEMS Microbiol Ecol* 91(7):iv067. <https://doi.org/10.1093/femsec/fiv067>
- Aldeguer-Riquelme B, Rubio-Portillo E, Álvarez-Rogel J, Giménez-Casaldueiro F, Otero XL, Belando M-D, Bernardeau-Esteller J, García-Muñoz R, Forcada A, Ruiz JM, Santos F, Antón J (2022) Factors structuring microbial communities in highly impacted coastal marine sediments (Mar Menor lagoon, SE Spain). *Front Microbiol*. <https://doi.org/10.3389/fmicb.2022.937683>
- Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA+ for primer: guide to software and statistical methods. PRIMER-E, Plymouth
- Andrew N, Viejo R (1998) Ecological limits to the invasion of *Sargassum muticum* in northern Spain. *Aquat Bot* 60:251–263. [https://doi.org/10.1016/S0304-3770\(97\)00088-0](https://doi.org/10.1016/S0304-3770(97)00088-0)
- Barbera P, Kozlov AM, Czech L, Morel B, Darriba D, Flouri T, Stamatakis A (2019) EPA-ng: massively parallel evolutionary placement of genetic sequences. *Syst Biol* 68:365–369. <https://doi.org/10.1093/sysbio/syy054>
- Bell JD, Steffe AS, Westoby M (1988) Location of seagrass beds in estuaries: effects on associated fish and decapods. *J Exp Mar Biol Ecol* 122:127–146. [https://doi.org/10.1016/0022-0981\(88\)90180-3](https://doi.org/10.1016/0022-0981(88)90180-3)
- Bernardeau-Esteller J, Ruiz JM, Tomas F, Sandoval-Gil JM, Marín-Guirao L (2015) Photoacclimation of *Caulerpa cylindracea*: Light as a limiting factor in the invasion of native Mediterranean seagrass meadows. *J Exp Mar Biol Ecol* 465:130–141. <https://doi.org/10.1016/j.jembe.2014.11.012>
- Bowman JP (2006) The marine clade of the family Flavobacteriaceae: the Genera Aequorivita, Arenibacter, Cellulophaga, Croceibacter, Formosa, Gelidibacter, Gillisia, Maribacter, Mesonia, Muricauda, Polaribacter, Psychroflexus, Psychroserpens, Robiginitalea, Salegentibacter, Tenacibaculum, Ulvibacter, Vitellibacter and Zobellia. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds) *The prokaryotes: volume 7: proteobacteria: delta, epsilon subclass*. Springer, Berlin, pp 677–694. https://doi.org/10.1007/0-387-30747-8_26
- Bulleri F, Tamburello L, Pusceddu A, Bonechi L, Cau A, Moccia D, Gribben PE (2018) Fragment quality and sediment organic loading regulate the survival of an invasive, clonal seaweed. *Biol Invasions* 20(8):1953–1959. <https://doi.org/10.1007/s10530-018-1685-4>
- Bulleri F, Marzinelli EM, Voerman SE, Gribben PE (2020) Propagule composition regulates the success of an invasive seaweed across a heterogeneous seascape. *J Ecol* 108:1061–1073. <https://doi.org/10.1111/1365-2745.13313>
- Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: high-resolution sample inference from Illumina amplicon data. *Nat Methods* 13:581–583. <https://doi.org/10.1038/nmeth.3869>
- Callaway RM, Ridenour WM (2004) Novel weapons: invasive success and the evolution of increased competitive ability. *Front Ecol Environ* 2(8):436–443. [https://doi.org/10.1890/1540-9295\(2004\)002\[0436:NWISAT\]2.0.CO;2](https://doi.org/10.1890/1540-9295(2004)002[0436:NWISAT]2.0.CO;2)
- Callaway RM, Thelen GC, Rodriguez A, Holben WE (2004) Soil biota and exotic plant invasion. *Nature* 427:731–733. <https://doi.org/10.1038/nature02322>
- Ceccherelli G, Cinelli F (1998) Habitat effect on spatio-temporal variability in size and density of the introduced alga *Caulerpa taxifolia*. *Mar Ecol Prog Ser* 163:289–294. <https://doi.org/10.3354/meps163289>
- Ceccherelli G, Piazza L, Cinelli F (2000) Response of the non-indigenous *Caulerpa racemosa* (Forsskål) J. Agardh to the native seagrass *Posidonia oceanica* (L.) Delile: effect of density of shoots and orientation of edges of meadows. *J Exp Mar Biol Ecol* 243:227–240. [https://doi.org/10.1016/S0022-0981\(99\)00122-7](https://doi.org/10.1016/S0022-0981(99)00122-7)
- Ceccherelli G, Pinna S, Cusceddu V, Bulleri F (2014) The role of disturbance in promoting the spread of the invasive seaweed *Caulerpa racemosa* in seagrass meadows. *Biol Invasions* 16:2737–2745. <https://doi.org/10.1007/s10530-014-0700-7>
- Chisholm JRM, Moulin P (2003) Stimulation of nitrogen fixation in refractory organic sediments by *Caulerpa taxifolia* (Chlorophyta). *Limnol Oceanogr* 48(2):787–794. <https://doi.org/10.4319/lo.2003.48.2.0787>
- Cipollini D, Rigsby CM, Barto EK (2012) Microbes as targets and mediators of allelopathy in plants. *J Chem Ecol* 38:714–727. <https://doi.org/10.1007/s10886-012-0133-7>
- Clark GF, Johnston EL (2009) Propagule pressure and disturbance interact to overcome biotic resistance of marine invertebrate communities. *Oikos* 118:1679–1686
- Clarke KR, Gorley RN (2006) Primer v6: user manual/tutorial. Primer E: Plymouth Marine Laboratory, Plymouth

- Creese RG, Davis AR, Glasby TM (2004) Eradicating and preventing the spread of the invasive alga *Caulerpa taxifolia* in NSW. NSW Fish Final Rep Ser 64:110
- Czech L, Barbera P, Stamatakis A (2020) Genesis and Gappa: processing, analyzing and visualizing phylogenetic (placement) data. *Bioinformatics* 36:3263–3265. <https://doi.org/10.1093/bioinformatics/btaa070>
- David P, Thebault E, Anneville O, Duyck PF, Chapuis E, Loeuille N (2017) Impacts of invasive species on food webs: a review of empirical data. *Adv Ecol Res* 56:1–60. <https://doi.org/10.1016/bs.aecr.2016.10.001>
- Davis MA, Grime JP, Thompson K (2000) Fluctuating resources in plant communities: a general theory of invasibility. *J Ecol* 88(3):528–534. <https://doi.org/10.1046/j.1365-2745.2000.00473.x>
- Davis NM, Proctor DM, Holmes SP, Relman DA, Callahan BJ (2018) Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. *Microbiome* 6:1–14. <https://doi.org/10.1186/s40168-018-0605-2>
- Dayton PK (1972) Toward an understanding of community resilience and the potential effects of enrichments to the benthos at McMurdo Sound, Antarctica. In: *Proceedings of the colloquium on conservation problems in Antarctica*. Lawrence, Kansas, USA: Allen Press, pp 81–96
- de Wit R (2008) Microbial diversity in the Bassin d'Arcachon coastal lagoon (SW France). *Hydrobiologia* 611(1):5–15. <https://doi.org/10.1007/s10750-008-9461-6>
- Douglas EJ, Pilditch CA, Lohrer AM, Savage C, Schipper LA, Thrush SF (2018) Sedimentary environment influences ecosystem response to nutrient enrichment. *Estuaries Coast* 41:1994–2008. <https://doi.org/10.1007/s12237-018-0416-5>
- Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, Huttenhower C, Langille MG (2020) PICRUSt2 for prediction of metagenome functions. *Nat Biotechnol* 38:685–688. <https://doi.org/10.1038/s41587-020-0548-6>
- Edgar RC, Flyvbjerg H (2015) Error filtering, pair assembly and error correction for next-generation sequencing reads. *Bioinformatics* 31:3476–3482. <https://doi.org/10.1093/bioinformatics/btv401>
- Elgersma KJ, Yu S, Vor T, Ehrenfeld JG (2012) Microbial-mediated feedbacks of leaf litter on invasive plant growth and interspecific competition. *Plant Soil* 356:341–355. <https://doi.org/10.1007/s11104-011-1117-z>
- Emery SM, Gross KL (2007) Dominant species identity, not community evenness, regulates invasion in experimental grassland plant communities. *Ecology* 88:954–964. <https://doi.org/10.1890/06-0568>
- Eyre BD, Maher D, Oakes JM, Erler DV, Glasby TM (2011) Differences in benthic metabolism, nutrient fluxes, and denitrification in *Caulerpa taxifolia* communities compared to uninvaded bare sediment and seagrass (*Zostera capricorni*) habitats. *Limnol Oceanogr* 56(5):1737–1750. <https://doi.org/10.4319/lo.2011.56.5.1737>
- Firth LB, Foggo A, Watts T, Knights AM, DeAmicis S (2024) Invasive macroalgae in native seagrass beds: vectors of spread and impacts. *Ann Bot* 133(1):41–50. <https://doi.org/10.1093/aob/mcad143>
- Fournier J, Gallon RK, Paris R (2014) G2Sd: a new R package for the statistical analysis of unconsolidated sediments. *Géomorphol Relief Process Environ* 20(1):73–78. <https://doi.org/10.4000/geomorphologie.10513>
- Fourqurean JW, Duarte CM, Kennedy H, Marbà N, Holmer M, Mateo MA, Apostolaki ET, Kendrick GA, Krause-Jensen D, McGlathery KJ, Serrano O (2012) Seagrass ecosystems as a globally significant carbon stock. *Nat Geosci* 5(7):505–509. <https://doi.org/10.1038/ngeo1477>
- Fox J, Weisberg S (2018) An R companion to applied regression, 3rd edn. SAGE Publications Inc., Thousand Oaks, pp 43–115
- Fuggle RE, Gribben PE, Marzinelli EM (2023) Experimental evidence root-associated microbes mediate seagrass response to environmental stress. *J Ecol* 111(5):1079–1093. <https://doi.org/10.1111/1365-2745.14081>
- Fukano Y, Tachiki Y, Yahara T, Iwasa Y (2013) Soil disturbances can suppress the invasion of alien plants under plant–soil feedback. *Ecol Model* 260:42–49. <https://doi.org/10.1016/j.ecolmodel.2013.03.022>
- Gallardo B, Clavero M, Sánchez MI, Vilà M (2016) Global ecological impacts of invasive species in aquatic ecosystems. *Glob Change Biol* 22(1):151–163. <https://doi.org/10.1111/gcb.13004>
- Gioria M, Osborne BA (2014) Resource competition in plant invasions: emerging patterns and research needs. *Front Plant Sci* 5:103442. <https://doi.org/10.3389/fpls.2014.00501>
- Glasby TM (2013) *Caulerpa taxifolia* in seagrass meadows: Killer or opportunistic weed? *Biol Invasions* 15:1017–1035. <https://doi.org/10.1007/s10530-012-0347-1>
- Glasby TM, West G (2018) Dragging the chain: Quantifying continued losses of seagrasses from boat moorings. *Aquat Conserv Mar Freshwat Ecosyst* 28(2):383–394. <https://doi.org/10.1002/aqc.2872>
- Gladstone-Gallagher RV, Pilditch CA, Stephenson F, Thrush SF (2019) Linking traits across ecological scales determines functional resilience. *Trends Ecol Evol* 34(12):1080–1091. <https://doi.org/10.1016/j.tree.2019.07.010>
- Gonzalez, SV, Johnston, EL, Dafforn, KA, O'Connor, WA, & Gribben, PE. (2021). Body size affects lethal and sublethal responses to organic enrichment: Evidence of associational susceptibility for an infaunal bivalve. *Marine Environmental Research*, 169, 105391. <https://doi.org/10.1016/j.marenvres.2021.105391>
- Gribben PE, Byers JE (2020) Comparative biogeography of marine invaders across their native and introduced ranges. *Oceanogr Mar Biol Annu Rev* 58:395–440
- Gribben PE, Byers JE, Wright JT, Glasby TM (2013) Positive versus negative effects of an invasive ecosystem engineer on different community components. *Oikos* 122:816–824. <https://doi.org/10.1111/j.1600-0706.2012.20868.x>
- Gribben PE, Nielsen S, Seymour JR, Bradley DJ, West MN, Thomas T (2017) Microbial communities in marine sediments modify success of an invasive macrophyte. *Sci Rep* 7(1):9845. <https://doi.org/10.1038/s41598-017-10231-2>
- Gribben PE, Thomas T, Pusceddu A, Bonechi L, Bianchelli S, Buschi E, Nielsen S, Ravaglioli C, Bulleri F (2018) Below-ground processes control the success of an

- invasive seaweed. *J Ecol* 106(5):2082–2095. <https://doi.org/10.1111/1365-2745.12966>
- Guido A, Pillar VD (2015) Are removal experiments effective tools for assessing plant community resistance and recovery from invasion? *J Veg Sci* 26(3):608–613. <https://doi.org/10.1111/jvs.12248>
- Habib C, Houel A, Lunazzi A, Bernardet J-F, Olsen AB, Nilsen H, Toranzo AE, Castro N, Nicolas P, Duchaud E (2014) Multilocus sequence analysis of the marine bacterial genus *Tenacibaculum* suggests parallel evolution of fish pathogenicity and endemic colonization of aquaculture systems. *Appl Environ Microbiol* 80(17):5503–5514. <https://doi.org/10.1128/AEM.01177-14>
- Hastings K, Hesp P, Kendrick GA (1995) Seagrass loss associated with boat moorings at Rottneest Island. *West Aust Ocean Coast Manag* 26(3):225–246. [https://doi.org/10.1016/0964-5691\(95\)00012-Q](https://doi.org/10.1016/0964-5691(95)00012-Q)
- Holle BV, Simberloff D (2005) Ecological resistance to biological invasion overwhelmed by propagule pressure. *Ecology* 86(12):3212–3218. <https://doi.org/10.1890/05-0427>
- Hollebone AL, Hay ME (2007) Population dynamics of the non-native crab *Petrolisthes armatus* invading the South Atlantic Bight at densities of thousands m⁻². *Mar Ecol Prog Ser* 336:211–223
- Holmer M, Marbà N, Lamote M, Duarte CM (2009) Deterioration of sediment quality in seagrass meadows (*Posidonia oceanica*) invaded by Macroalgae (*Caulerpa* sp.). *Estuar Coasts* 32(3):456–466. <https://doi.org/10.1007/s12237-009-9133-4>
- Hyland J, Balthis L, Karakassis I, Magni P, Petrov A, Shine J, Vestergaard O, Warwick R (2005) Organic carbon content of sediments as an indicator of stress in the marine benthos. *Mar Ecol Prog Ser* 295:91–103
- Iponga DM, Milton SJ, Richardson DM (2008) Superiority in competition for light: a crucial attribute defining the impact of the invasive alien tree *Schinus molle* (Anacardiaceae) in South African savanna. *J Arid Environ* 72:612–623. <https://doi.org/10.1016/j.jaridenv.2007.10.001>
- Jeschke J, Aparicio LG, Haider S, Heger T, Lortie C, Pyšek P, Strayer D (2012) Support for major hypotheses in invasion biology is uneven and declining. *NeoBiota* 14:1–20. <https://doi.org/10.3897/neobiota.14.3435>
- Jiang J, Abbott KC, Baudena M, Eppinga MB, Umbanhowar JA, Bever JD (2020) Pathogens and mutualists as joint drivers of host species coexistence and turnover: implications for plant competition and succession. *Am Nat* 195(4):591–602. <https://doi.org/10.1086/707355>
- Klironomos JN (2002) Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature* 417(6884):67–70. <https://doi.org/10.1038/417067a>
- Kominoski JS, Gaiser EE, Castañeda-Moya E, Davis SE, Dessu SB, Julian P, Lee DY, Marazzi L, Rivera-Monroy VH, Sola A, Stingl U, Stumpf S, Surratt D, Travieso R, Troxler TG (2020) Disturbance legacies increase and synchronize nutrient concentrations and bacterial productivity in coastal ecosystems. *Ecology* 101(5):e02988. <https://doi.org/10.1002/ecy.2988>
- Korlević M, Markovski M, Zhao Z, Herndl GJ, Najdek M (2021) Seasonal dynamics of epiphytic microbial communities on marine macrophyte surfaces. *Front Microbiol* 12:671342. <https://doi.org/10.3389/fmicb.2021.671342>
- Kourtev PS, Ehrenfeld JG, Haggblom M (2003) Experimental analysis of the effect of exotic and native plant species on the structure and function of soil microbial communities. *Soil Biol Biochem* 35:895–905. [https://doi.org/10.1016/S0038-0717\(03\)00120-2](https://doi.org/10.1016/S0038-0717(03)00120-2)
- Kraan C, Thrush SF, Dormann CF (2020) Co-occurrence patterns and the large-scale spatial structure of benthic communities in seagrass meadows and bare sand. *BMC Ecol* 20(1):37. <https://doi.org/10.1186/s12898-020-00308-4>
- Lane D (1991) 16S/23S rRNA sequencing. *Nucleic acid techniques in bacterial systematics*. John Wiley and Sons, New York, pp 115–175
- Langille MG, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepille DE, Vega Thurber RL, Knight R (2013) Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat Biotechnol* 31:814–821. <https://doi.org/10.1038/nbt.2676>
- Leung B, Mandrak NE (2007) The risk of establishment of aquatic invasive species: joining invasibility and propagule pressure. *Proc R Soc B Biol Sci* 274:2603–2609. <https://doi.org/10.1098/rspb.2007.0841>
- Lim SJ, Alexander L, Engel AS, Paterson AT, Anderson LC, Campbell BJ (2019) Extensive thioautotrophic gill endosymbiont diversity within a single *Ctena orbiculata* (Bivalvia: Lucinidae) population and implications for defining host-symbiont specificity and species recognition. *Msystems* 4(4):10–1128. <https://doi.org/10.1128/msystems.00280-19>
- Ling J, Zhou W, Yang Q, Yin J, Zhang J, Peng Q, Huang X, Zhang Y, Dong J (2021) Spatial and species variations of bacterial community structure and putative function in seagrass rhizosphere sediment. *Life* 11(8):852. <https://doi.org/10.3390/life11080852>
- Lockwood JL, Hoopes MF, Marchetti MP (2013) *Invasion ecology*, 2nd edn. Wiley-Blackwell Publishers, New Jersey, pp 74–97
- López-Sánchez R, Rebollar EA, Gutiérrez-Ríos RM, Garcíarrubio A, Juárez K, Segovia L (2024) Metagenomic analysis of carbohydrate-active enzymes and their contribution to marine sediment biodiversity. *World J Microbiol Biotechnol* 40(3):95. <https://doi.org/10.1007/s11274-024-03884-5>
- Louca S, Doebeli M (2018) Efficient comparative phylogenetics on large trees. *Bioinformatics* 34:1053–1055. <https://doi.org/10.1093/bioinformatics/btx701>
- Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 15:1–21. <https://doi.org/10.1186/s13059-014-0550-8>
- Lüdecke D, Ben-Shachar MS, Patil I, Waggoner P, Makowski D (2021) performance: an R package for assessment, comparison and testing of statistical models. *J Open Sour Softw* 6:3139
- Mangla, S & Callaway, RM. (2008). Exotic invasive plant accumulates native soil pathogens which inhibit native plants. *Journal of Ecology*, 96(1):58–67. <https://doi.org/10.1111/j.1365-2745.2007.01312.x>

- Martin BC, Alarcon MS, Gleeson D, Middleton JA, Fraser MW, Ryan MH, Holmer M, Kendrick GA, Kilminster K (2020a) Root microbiomes as indicators of seagrass health. *FEMS Microbiol Ecol* 96(2):fiz201. <https://doi.org/10.1093/femsec/fiz201>
- Martin BC, Middleton JA, Fraser MW, Marshall IP, Scholz VV, Hausl B, Schmidt H (2020b) Cutting out the middle clam: lucinid endosymbiotic bacteria are also associated with seagrass roots worldwide. *ISME J* 14:2901–2905. <https://doi.org/10.1038/s41396-020-00771-3>
- McGlathery KJ, Risgaard-Petersen N, Christensen PB (1998) Temporal and spatial variation in nitrogen fixation activity in the eelgrass *Zostera marina* rhizosphere. *Mar Ecol Prog Ser* 168:245–258. <https://doi.org/10.3354/meps168245>
- McKinnon JG, Gribben PE, Davis AR, Jolley DF, Wright JT (2009) Differences in soft-sediment macrobenthic assemblages invaded by *Caulerpa taxifolia* compared to uninvaded habitats. *Mar Ecol Prog Ser* 380:59–71. <https://doi.org/10.3354/meps07926>
- McMurdie PJ, Holmes S (2013) phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE* 8:e61217. <https://doi.org/10.1371/journal.pone.0061217>
- Miranda K, Weigel BL, Fogarty EC, Veseli IA, Giblin AE, Eren AM, Pfister CA (2022) The diversity and functional capacity of microbes associated with coastal macrophytes. *mSystems* 7(5):e00592–e622. <https://doi.org/10.1128/msystems.00592-22>
- Murcia C (1995) Edge effects in fragmented forests: implications for conservation. *Trends Ecol Evol* 10:58–62. [https://doi.org/10.1016/S0169-5347\(00\)88977-6](https://doi.org/10.1016/S0169-5347(00)88977-6)
- Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'hara R, Simpson GL, Solyomos P (2019) Package 'vegan'. Community ecology package, version, 2. <http://CRAN.R-project.org/package=vegan>
- Parks DH, Chuvochina M, Rinke C, Mussig AJ, Chaumeil P-A, Hugenholtz P (2021) GTDB: an ongoing census of bacterial and archaeal diversity through a phylogenetically consistent, rank normalized and complete genome-based taxonomy. *Nucleic Acids Res* 50:D785–D794. <https://doi.org/10.1093/nar/gkab776>
- Piazzi L, Balata D, Ceccherelli G, Cinelli F (2005) Interactive effect of sedimentation and *Caulerpa racemosa* var. *cylindracea* invasion on macroalgal assemblages in the Mediterranean Sea. *Estuar Coast Shelf Sci* 64:467–474. <https://doi.org/10.1016/j.ecss.2005.03.010>
- Prevéy JS, Germino MJ, Huntly NJ (2010) Loss of foundation species increases population growth of exotic forbs in sagebrush steppe. *Ecol Appl* 20(7):1890–1902. <https://doi.org/10.1890/09-0750.1>
- Ravaglioli C, De Marchi L, Anselmi S, Dattolo E, Fontanini D, Pretti C, Procaccini G, Rilov G, Renzi M, Silverman J, Bulleri F (2024) Ocean acidification impairs seagrass performance under thermal stress in shallow and deep water. *Environ Res* 241:117629. <https://doi.org/10.1016/j.envres.2023.117629>
- Ricciardi A, Palmer ME, Yan ND (2011) Should biological invasions be managed as natural disasters? *BioScience* 61(4):312–317. <https://doi.org/10.1525/bio.2011.61.4.11>
- Romanuk TN, Kolasa J (2005) Resource limitation, biodiversity, and competitive effects interact to determine the invasibility of rock pool microcosms. *Biol Invasions* 7:711–722. <https://doi.org/10.1007/s10530-004-0997-8>
- Saha M, Weinberger F (2019) Microbial “gardening” by a seaweed holobiont: surface metabolites attract protective and deter pathogenic epibacterial settlement. *J Ecol* 107:2255–2265. <https://doi.org/10.1111/1365-2745.13193>
- Salgado J, Sayer CD, Willby N, Baker AG, Goldsmith B, McGowan S, Davidson TA, Bexell P, Patmore IR, Okamura B (2022) Habitat heterogeneity enables spatial and temporal coexistence of native and invasive macrophytes in shallow lake landscapes. *River Res Appl* 38(8):1387–1399. <https://doi.org/10.1002/rra.3839>
- Scheibling RE, Gagnon P (2006) Competitive interactions between the invasive green alga *Codium fragile* ssp. *tomentosoides* and native canopy-forming seaweeds in Nova Scotia (Canada). *Mar Ecol Prog Ser* 325:1–14
- Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* 9:671–675. <https://doi.org/10.1038/nmeth.2089>
- Seymour JR, Laverock B, Nielsen DA, Trevathan-Tackett SM, Macreadie PI (2018) The microbiology of seagrasses. In: Larkum AWD, Kendrick GA, Ralph PJ (eds) *Seagrasses of Australia*. Springer International Publishing, Berlin, pp 343–392. https://doi.org/10.1007/978-3-319-71354-0_12
- Short FT, Wyllie-Echeverria S (1996) Natural and human-induced disturbance of seagrasses. *Environ Conserv* 23(1):17–27. <https://doi.org/10.1017/S0376892900038212>
- Song X, Zeng J, Zhou Y, Chen Q, Yang H, Shou L, Liao Y, Huang W, Du P, Liu Q (2021) Partial function prediction of sulfate-reducing bacterial community from the rhizospheres of two typical coastal wetland plants in China. *J Oceanol Limnol* 39(1):185–197. <https://doi.org/10.1007/s00343-019-9177-7>
- Sorokin DY, Tourova TP, Braker G, Muyzer G (2007) *Thiohalomonas denitrificans* gen. Nov., sp. Nov. and *Thiohalomonas nitratreducens* sp. Nov., novel obligately chemolithoautotrophic, moderately halophilic, thiodenitrifying Gammaproteobacteria from hypersaline habitats. *Int J Syst Evolut Microbiol* 57(7):1582–1589. <https://doi.org/10.1099/ijs.0.65112-0>
- Statton J, Gustin-Craig S, Dixon KW, Kendrick GA (2015) Edge effects along a seagrass margin result in an increased grazing risk on *Posidonia australis* transplants. *PLoS ONE* 10:e0137778. <https://doi.org/10.1371/journal.pone.0137778>
- Steen H (2003) Intraspecific competition in *Sargassum muticum* (Phaeophyceae) germlings under various density, nutrient and temperature regimes. *Bot Mar* 46(1):36–43. <https://doi.org/10.1515/BOT.2003.006>
- Thomas F, Giblin AE, Cardon ZG, Sievert SM (2014) Rhizosphere heterogeneity shapes abundance and activity of sulfur-oxidizing bacteria in vegetated salt marsh sediments. *Front Microbiol* 5:96784. <https://doi.org/10.3389/fmicb.2014.00309>
- Uyà M, Bulleri F, Gribben PE (2018) Propagules are not all equal: traits of vegetative fragments and disturbance

- regulate invasion success. *Ecology* 99(4):957–965. <https://doi.org/10.1002/ecy.2168>
- Valentine JP, Johnson CR (2003) Establishment of the introduced kelp *Undaria pinnatifida* in Tasmania depends on disturbance to native algal assemblages. *J Exp Mar Biol Ecol* 295:63–90. [https://doi.org/10.1016/S0022-0981\(03\)00272-7](https://doi.org/10.1016/S0022-0981(03)00272-7)
- Valentine JP, Johnson CR (2004) Establishment of the introduced kelp *Undaria pinnatifida* following dieback of the native macroalga *Phyllospora comosa* in Tasmania, Australia. *Mar Freshw Res* 55:223–230. <https://doi.org/10.1071/MF03048>
- Van der Putten WH, Van Dijk C, Peters BAM (1993) Plant-specific soil-borne diseases contribute to succession in foredune vegetation. *Nature* 362(6415):53–56. <https://doi.org/10.1038/362053a0>
- Van der Putten WH, Klironomos JN, Wardle DA (2007) Microbial ecology of biological invasions. *ISME J* 1:28–37. <https://doi.org/10.1038/ismej.2007.9>
- Van der Putten WH, Bradford MA, Pernilla Brinkman E, van de Voorde TF, Veen GF (2016) Where, when and how plant–soil feedback matters in a changing world. *Funct Ecol* 30(7):1109–1121.
- Walker LDA, Gribben PE, Glasby T, Marzinelli EM, Varkey D, Dafforn KA. (2024) Above and below-ground bacterial communities shift in seagrass beds with warmer temperatures. *Frontiers in Marine Science*.
- Wang Q, Li Y, Wang Y (2011) Optimizing the weight loss-on-ignition methodology to quantify organic and carbonate carbon of sediments from diverse sources. *Environ Monit Assess* 174:241–257. <https://doi.org/10.1007/s10661-010-1454-z>
- Wang Y, Naumann U, Wright ST, Warton DI (2012) mvabund—an R package for model-based analysis of multivariate abundance data. *Methods Ecol Evol* 3:471–474. <https://doi.org/10.1111/j.2041-210X.2012.00190.x>
- Wei T, Simko V, Levy M, Xie Y, Jin Y, Zemla J (2013) CorrPlot: visualization of a correlation matrix. R package version 0.73, 230. <https://github.com/taiyun/corrplot>
- West R (2012) Impacts of recreational boating activities on the seagrass *Posidonia* in SE Australia. *Wetl Austral J* 26(2):3–13
- West EJ, Barnes PB, Wright JT, Davis AR (2007) Anchors aweigh: fragment generation of invasive *Caulerpa taxifolia* by boat anchors and its resistance to desiccation. *Aquat Bot* 87(3):196–202. <https://doi.org/10.1016/j.aquabot.2007.06.005>
- West EJ, Davis AR, Barnes PB, Wright JT (2009) The role of recreational activities in creating fragments of invasive *Caulerpa taxifolia*. *J Exp Mar Biol Ecol* 376(1):17–25. <https://doi.org/10.1016/j.jembe.2009.05.015>
- Wheeler TJ, Eddy SR (2013) nhmmer: DNA homology search with profile HMMs. *Bioinformatics* 29:2487–2489. <https://doi.org/10.1093/bioinformatics/btt403>
- Wickham H, Chang W, Wickham MH (2016) Package ‘ggplot2’. Create elegant data visualisations using the grammar of graphics. Version, 2, pp 1–189. <https://ggplot2.tidyverse.org/reference/ggplot2-package.html#author>
- Wolfe BE, Klironomos JN (2005) Breaking new ground: soil communities and exotic plant invasion. *Bioscience* 55:477–487. [https://doi.org/10.1641/0006-3568\(2005\)055\[0477:BNGSCA\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2005)055[0477:BNGSCA]2.0.CO;2)
- Wright JT, Davis AR (2006) Demographic feedback between clonal growth and fragmentation in an invasive seaweed. *Ecology* 87(7):1744–1754. [https://doi.org/10.1890/0012-9658\(2006\)87\[1744:DFBCGA\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2006)87[1744:DFBCGA]2.0.CO;2)
- Ye Y, Doak TG (2009) A parsimony approach to biological pathway reconstruction/inference for genomes and metagenomes. *PLoS Comput Biol* 5:e1000465. <https://doi.org/10.1371/journal.pcbi.1000465>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.