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The invasive seaweed Asparagopsis taxiformis erodes the habitat structure and biodiversity of native algal forests in the Mediterranean Sea

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Marine Environmental Research

The invasive seaweed Asparagopsis taxiformis erodes the habitat structure and biodiversity of native algal forests in the Mediterranean Sea

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Abstract:	Invasive seaweeds are listed among the most relevant threats to marine ecosystems worldwide. Biodiversity hotspots, such as the Mediterranean Sea, are facing multiple invasions and are expected to be severely affected by the introduction of new non-native seaweeds in the near future. In this study, we evaluated the consequences of the shift from the native Ericaria brachycarpa to the invasive Asparagopsis taxiformis habitat on the shallow rocky shores of Favignana Island (Egadi Islands, MPA, Sicily, Italy). We compared algal biomass and species composition and structure of the associated epifaunal assemblages in homogenous and mixed stands of E. brachycarpa and A. taxiformis . The results showed that the biomass of primary producers is reduced by 90% in the A. taxiformis invaded habitat compared to the E. brachycarpa native habitat. The structure of the epifaunal assemblages displayed significant variations among homogenous and mixed stands. The abundance, species richness and Shannon-Wiener diversity index of the epifaunal assemblages decreased by 89%, 78% and 40%, respectively, from homogenous stands of the native E. brachycarpa to the invasive A. taxiformis . Seaweed biomass was the structural attribute better explaining the variation in epifaunal abundance, species richness and diversity. Overall, our results suggest that the shift from E. brachycarpa to A. taxiformis habitat would drastically erode the biomass of primary producers and the associated biodiversity. We hypothesize that a complete shift from native to invasive seaweeds could ultimately lead to bottom-up effects on rocky shore habitats, with negative consequences for the ecosystem structure, functioning, and the services provided.
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Highlights:

- Primary producer biomass is 90% lower in *A. taxiformis* than in *E. brachycarpa* habitat.
- From native to invasive seaweeds, epifaunal assemblages lost 40% of their diversity.
- Seaweed biomass was the structural attribute explaining the epifaunal variation.
- We suggest a complete shift from native to invasive seaweeds could impair rocky shore habitats.

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The invasive seaweed *Asparagopsis taxiformis* erodes the habitat structure and biodiversity of native algal forests in the Mediterranean Sea

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Abstract

Invasive seaweeds are listed among the most relevant threats to marine ecosystems worldwide. Biodiversity hotspots, such as the Mediterranean Sea, are facing multiple invasions and are expected to be severely affected by the introduction of new non-native seaweeds in the near future. In this study, we evaluated the consequences of the shift from the native *Ericaria brachycarpa* to the invasive *Asparagopsis taxiformis* habitat on the shallow rocky shores of Favignana Island (Egadi Islands, MPA, Sicily, Italy). We compared algal biomass and species composition and structure of

the associated epifaunal assemblages in homogenous and mixed stands of *E. brachycarpa* and *A. taxiformis*. The results showed that the biomass of primary producers is reduced by 90% in the *A. taxiformis* invaded habitat compared to the *E. brachycarpa* native habitat. The structure of the epifaunal assemblages displayed significant variations among homogenous and mixed stands. The abundance, species richness and Shannon-Wiener diversity index of the epifaunal assemblages decreased by 89%, 78% and 40%, respectively, from homogenous stands of the native *E. brachycarpa* to the invasive *A. taxiformis*. Seaweed biomass was the structural attribute better explaining the variation in epifaunal abundance, species richness and diversity. Overall, our results suggest that the shift from *E. brachycarpa* to *A. taxiformis* habitat would drastically erode the biomass of primary producers and the associated biodiversity. We hypothesize that a complete shift from native to invasive seaweeds could ultimately lead to bottom-up effects on rocky shore habitats, with negative consequences for the ecosystem structure, functioning, and the services provided.

Keywords: habitat shift; alga; *Ericaria brachycarpa*; epifauna diversity; *Cystoseira sensu lato*; rocky shore; Marine Protected Area

Introduction

Invasive species are globally recognized among the main drivers of habitat shift in both terrestrial and marine ecosystems (Gallardo et al., 2016; Mačić et al., 2018). In marine environments, decades of human activities related to global aquatic trade have enabled the dispersion of invasive species among distant geographic areas worldwide (Bax et al., 2003; Williams and Smith, 2007; Molnar et al., 2008; Servello et al., 2019). In addition, the rise in seawater temperature caused by global warming has allowed non-native species to cross environmental and geographical barriers, facilitating their expansion and in turn eroding indigenous resistance (Occhipinti-Ambrogi and Galil, 2010; Lo Brutto et al., 2019). Concerns over ecological and social-economic consequences have led researches to investigate the effects of invasive species on many marine ecosystems around the world.

Seaweeds are a significant component of marine non-native and invasive species (227 taxa globally), with some of them being responsible for drastic habitat shifts (Williams and Smith, 2007). Studies have highlighted how invasive seaweeds can negatively impact the recipient habitats by reducing biomass of primary producers, biodiversity, and nutrient flows, compromising ecosystem functioning (Boudouresque et al., 2005; Streftaris and Zenetos, 2006; Thomsen et al., 2014; Maggi et al., 2015; Ramsay-Newton et al., 2017; Geburzi and McCarthy, 2018). Interestingly, the effects of non-native seaweeds seem to change depending on the complexity of the recipient habitat (Thomsen et al., 2014). In less structured habitats (e.g. soft bottoms), the introduction of non-native seaweeds enhances structural complexity that may favor the increase in biodiversity and food web length (Dijkstra et al., 2017). Conversely, the introduction in well-structured habitats (e.g. seagrass meadows, algal canopies) may alter the diversity and function, depending on the structural features of the recipient habitat (Engelen et al., 2013; Veiga et al., 2014, 2018).

Macroalgal complexity plays a significant role in shaping the abundance, richness and structure of epifaunal assemblages associated with both non-native and native seaweeds (Chemello and Milazzo, 2002; Jormalainen and Honkanen, 2008; Pitacco et al., 2014; Veiga et al., 2014; Maggi et al., 2015; Dijkstra et al., 2017; Veiga et al., 2018; Chiarore et al., 2019). Algae with a high structural complexity, expressed as a combination of attributes, such as degree of branching, thallus width and height, and wet weight, can support well-structured epifaunal communities (Hacker and Steneck, 1990; Chemello and Milazzo, 2002; Jormalainen and Honkanen, 2008; Pitacco et al., 2014; Veiga et al., 2014; Bitlis, 2019; Chiarore et al., 2019). Studies comparing the epifaunal diversity between invasive and native seaweeds revealed that, when invasive species are structurally less complex than native ones, they support low abundance and richness, and a simplified structure of epifaunal assemblages (Navarro-Barranco et al., 2018; Veiga et al., 2018). However, when native macroalgae are less complex, the abundance and diversity of epifauna associated with invasive seaweeds may be higher (Veiga et al., 2014; Dijkstra et al., 2017). This indicates that the effects of invasive seaweeds on epifaunal assemblages may change depending on both the invasive and the native seaweed

structural features. However, other studies have shown that native and invasive seaweeds with similar morphologies can host either similar (Suárez-Jiménez et al., 2017) or different epifaunal diversity (Navarro-Barranco et al., 2019). Overall, these contrasting evidences suggest that, apart from seaweed morphology, other factors can be involved in structuring the associated assemblages.

In the last decades, the Mediterranean basin has witnessed an increase in the number of non-native and invasive seaweeds, with consequent modification of biodiversity and ecosystem functioning of coastal areas (Ribera Siguan, 2002; Streftaris et al., 2005; Streftaris and Zenetos, 2006; Piazzi and Balata, 2009; Musco et al., 2014; Bulleri et al., 2016; Corriero et al., 2016; Giangrande et al., 2020). In particular, *Asparagopsis taxiformis* (Delile) Trevisan de Saint-Léon is listed among the 100 worst invasive species in this basin (Streftaris and Zenetos, 2006). The earliest reports of its presence in the Mediterranean Sea date back to 1798-1801 in Alexandria (Egypt) as a result of trading operations and the opening of the Suez Canal (Verlaque et al., 2015). It was first recorded along the Italian coast on the western shore of Sicily, close to the city of Trapani, in May 2000 (Barone et al., 2003). While *A. taxiformis* has been suspected of producing harmful effects on native habitats (Barone et al., 2003), as far as we know, only one study assessed its effects on biodiversity, in particular by comparing the vagile macrofauna associated with this species to that of the native *Halopteris scoparia* (Linnaeus) Sauvageau indicating that *A. taxiformis* hosted less diverse epifaunal assemblages compared to native algae (Navarro-Barranco et al., 2018).

Along the Italian coast, gametophytes of *A. taxiformis* can colonize coastal areas dominated by habitat-forming seaweeds, mainly belonging to the genus *Cystoseira* - recently divided into three genera *Cystoseira*, *Gongolaria* and *Ericaria* and hereafter referred to as *Cystoseira sensu lato* to include all three genera (Orellana et al., 2019; Molinari Novoa and Guiry, 2020). *Cystoseira sensu lato* species are important ecological engineers, greatly increasing the habitat surface, complexity and productivity in coastal ecosystems from the infra-littoral zone to the upper circalittoral zone. (Giaccone et al., 1994; Bulleri et al., 2002; Falace and Bressan, 2006; Ballesteros et al., 2009; Mancuso et al., 2021b). By creating shelter, *Cystoseira sensu lato* species improve the biodiversity

of their related assemblages, leading to the development of well-structured food webs (Schiel and Foster, 2006; Cheminée et al., 2013; Mineur et al., 2015; Mancuso et al., 2021b). According to the European Water Framework Directive (2000/60), they are also considered indicators of good water and environmental quality (European Commission, 2000).

In a recent study, we discovered that *A. taxiformis* had a less diverse and less structured molluscan assemblage than *E. brachycarpa*; however, no information about other epifaunal components associated with seaweeds was reported, nor information about intermediate states, such as mixed stands of *E. brachycarpa* and *A. taxiformis* (Mancuso et al., 2021a). Here, we compared the structure of the epifaunal community (amphipods, molluscs and annelids) associated with three plausible alternative states of the transition between the native *Ericaria brachycarpa* (J.Agardth) Orellana & Sansón and the invasive *A. taxiformis*. In particular, we characterized and compared the biomass and the diversity (richness, evenness, structure and composition) of the epifauna associated with the fronds of homogenous and mixed stands of *E. brachycarpa* and *A. taxiformis*. Moreover, we explored the variation of the epifaunal diversity in relation to the structural features of the two algae (dry weight, thallus volume, canopy volume, and interstitial volume). We hypothesize that shifting from habitats dominated by *E. brachycarpa* to those dominated by *A. taxiformis* will have a negative impact on the associated biodiversity, resulting in low abundances and diversity of each epifaunal component.

Materials and Methods

Study area and algal species characteristics

The research was performed on the southwestern, shallow rocky shore of Favignana Island (Sicily, Italy), within the Egadi Islands Marine Protected Area (MPA) in June 2011 (Fig. 1). The region consists of gently sloping (5°-10°) carbonate rocky platforms and scattered boulders (Pepe et al., 2018) that provide substrates for well-developed macroalgal vegetation.

In this area, *A. taxiformis* was first recorded in 2000 (Barone et al., 2003). Since then, no studies have explored the temporal effects of this invasive species on native habitats. Although today *A. taxiformis* is well established in the area, previous surveys allowed the identification of three sites with distinctive habitats corresponding to three possible alternative states of the transition from native to invasive seaweed habitats: "Scoglio Corrente" (37° 55' 2.0778" N, 12° 17' 6.0432" E) characterized by stands of *E. brachycarpa* (100% coverage); "Scoglio Palumbo" (37° 55' 10.4226" N, 12° 18' 41.097" E) hosting stands of *A. taxiformis* (100% coverage), and "Cala Grande" (37° 55' 35.385" N, 12° 16' 39.514" E) with mixed stands of *E. brachycarpa* (~50% coverage) and *A. taxiformis* (~50% coverage) (Fig. 1). In this study, we decided to use these three sites to compare the epifaunal communities associated with three plausible alternative states of the transition from native to invasive habitats.

Ericaria brachycarpa is a brown seaweed (Fucales) characterized by caespitosus thalli up to 20-25 cm in height with several perennial axes, up to 2-6 cm in height, connected to the substratum by a more or less compact discoid base formed by haptera (Molinari Novoa and Guiry, 2020). The apices of the axes are flattened, smooth and not very prominent. Branches are cylindrical with smooth bases, or covered with tiny spinose appendages that are typically fertile in the spring-summer season (Gómez-Garreta et al., 2002; Mannino and Mancuso, 2009; Cormaci et al., 2012). Like other *Cystoseira sensu lato* species, *E. brachycarpa* displays seasonal differences in vegetative development (Gómez-Garreta et al., 2002). At the study sites, new branches of *E. brachycarpa* grow from the perennial axes in spring (May-June) providing new substrate and shelter for colonizing fauna, while in autumn (September-October) *E. brachycarpa* starts to become quiescent, losing almost all branches, leaving perennial axes that persist throughout the cold winter season.

Asparagopsis taxiformis is a red alga (Bonnemaisoniales) common in the tropics and subtropics across the globe. The species experiences a heteromorphic life cycle, with an erect gametophyte alternating with a filamentous sporophyte known as *Falkenbergia hillebrandii* (Bornet) Falkenberg (Andreakis et al., 2004; Ní Chualáin et al., 2004). The gametophytes are characterized by sparsely

branched, creeping stolons and erect shoots from which several side branches grow in all directions. The latter ramifies over and over again giving the thallus a plumose appearance. In the study area, *A. taxiformis* develops in the upper sublittoral zone of the rocky substrate or as an epiphyte of other algal species. The gametophytes are present during all seasons with a maximum occurrence in spring (Barone et al., 2003).

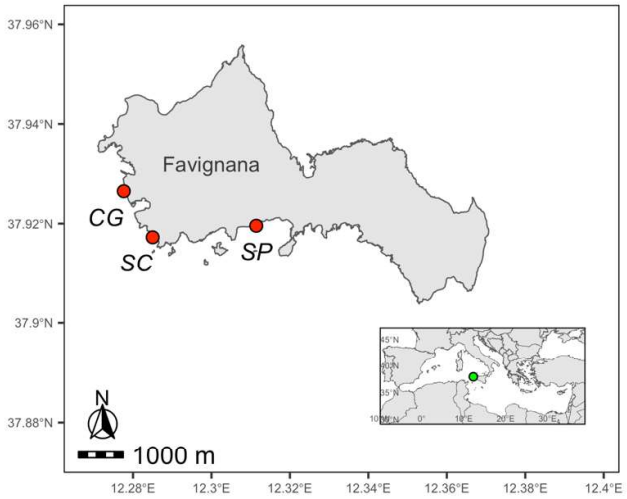

Fig. 1 Location of the three study sites (red dots) along the rocky-shore of Favignana Island MPA (green dot), Trapani, Sicily, Italy. **SC** = Scoglio Corrente, **SP** = Scoglio Palumbo, **CG** = Cala Grande.

Sampling and analysis of epifauna

Samples were collected by scuba diving at a depth of 5-7 m. For each site (hereafter referred to as habitat), two areas (5 x 5 m) were haphazardly selected. For each area, 10 thalli of *E. brachycarpa* from homogenous stands (100% algal coverage), 10 thalli of *E. brachycarpa* from mixed stands and 10 gametophytes of *A. taxiformis* from homogenous stands (100% algal coverage) were collected (n = 20 per habitat). Thalli were collected 50cm apart to avoid spatial autocorrelation among samples. Underwater, each thallus and the associated epifauna were enveloped in a plastic bag, then the alga was detached from the substrate and the plastic bag was immediately closed to prevent the escape of vagile fauna. After collection, each sample was carefully drained of seawater in order to prevent

escape of small epifauna and stored at -20°C until laboratory analysis. In the laboratory, each thallus of *E. brachycarpa* and gametophytes of *A. taxiformis* were transferred into buckets abundantly rinsed under running water, allowing the associated fauna to detach from the algae. Then, the water was sieved through a 1 mm mesh. After sorting, molluscs, amphipods, and annelids were stored in 70% seawater ethanol solution and subsequently counted and identified to species, or the nearest possible taxonomic level. Taxonomy and nomenclature were updated according to the World Register of Marine Species database (WoRMS Editorial Board (2021)).

Seaweed structural attributes

For each thallus of *E. brachycarpa* and gametophyte of *A. taxiformis* collected, we measured 4 structural features (thallus volume, canopy volume, interstitial volume, and biomass), to explore their relationships with the diversity indices calculated for the epifaunal assemblages. Thallus volume was measured as the variation of volume, in ml, after the immersion of a thallus into a graduated cylinder filled with seawater. Canopy and interstitial volumes were estimated according to Hacker and Steneck (1990). The canopy volume (*CV*, the volume, in ml, created by the overall dimension of a thallus submerged in seawater) was defined as the volume of a theoretical cylinder ($CV = \pi \times r^2 \times h$), where π = 3.14, *h* is the length of the thallus from the base to its apical portion of the thallus, including epiphytes, and *r* is the radius calculated as an average measure of the radius of the thallus measured with a ruler (+/- 1 mm) at the apical, median, and basal parts. The interstitial volume (*IV*, the volume, in ml, of water among the branches of the alga) was obtained by subtracting the thallus volume (*TV*) from the canopy volume *CV* ($IV = CV - TV$).

Finally, the biomass of the macroalgae was calculated as dry weight (*DW*, gr) after drying in a stove at 60 °C for 48 h (Stein-Taylor et al., 1985). Biomass was used as a proxy for the primary production of each habitat.

Data analysis

For each epifaunal species, we calculated total abundance (N), frequency (F%; the percentage of samples in which a particular species is present) and dominance index (D%; the percentage of the rate between the percentage of individuals of a particular species and the total number of individuals within the sample) (Magurran, 1988). The epifaunal assemblages of each habitat were characterized according to total abundance of individuals (N), rarefied species richness (S), Shannon-Wiener diversity index (H') and Pielou's Evenness index (J). A two-way analysis of variance (ANOVA) was used to test differences in the epifaunal indices (N, S, H', J) between habitats (fixed with 3 levels: *E. brachycarpa, E. brachycarpa* in mixed stands and *A. taxiformis*) and areas (random and nested within habitat with 2 levels: area 1 and area 2). Cochran's test was used to check for the homogeneity of variances (Underwood 1997). Tukey's HSD procedure was used to separate means (at $\alpha = 0.05$) following significant effects in the ANOVAs (Underwood, 1996). The hierarchical structure of the taxonomic classifications of the epifaunal assemblages of *E. brachycarpa, E. brachycarpa* in mixed stands, and *A. taxiformis* was visualized using the "heat_tree" function in the "Metacoder" R- package (Foster et al., 2017).

SIMPER analysis (Clarke, 1993) was performed to identify those taxa that contributed to the dissimilarity of the epifaunal assemblages between habitats ($\delta i\%$). The ratio $\delta i/SD_{(\delta i)}$ was used to measure the consistency of the contribution of a particular taxon to the average dissimilarity in the comparison between habitats. A cut-off value of 70% was used to exclude low contributions.

Differences in the epifaunal community structure (which takes into account species identity and relative abundance) and composition (presence/absence, which only takes into account species identity) among habitats and areas were assessed by Permutational Multivariate Analysis of Variance (PERMANOVA). The analyses were based on a Bray-Curtis distance matrix of square-root transformed epifaunal abundances (structure) and on a Jaccard distance matrix of presence/absence data (composition) using 9999 permutations. PERMANOVA was also performed separately for each component of the epifauna (molluscs, annelids and amphipods). Permutational analysis of multivariate dispersion (PERMDISP) was used to test differences in multivariate dispersion

(Anderson et al., 2008). A principal coordinate analysis (PCoA) plot was generated to visualize the variation of the epifaunal community structure (based on a Bray-Curtis distance matrix) and composition (based on a Jaccard distance matrix).

Differences in each of the structural attributes (*CV*, *IV*, *TV*, *DW*) among habitats and areas were analyzed by two-way ANOVAs according to the above mentioned design. Cochran's test was used to check for the homogeneity of variances (Underwood, 1996).

Linear regression (LM) analysis was used to test which algal structural attributes explained better the variation of total abundance (N), rarefied species richness (S), Shannon-Wiener diversity (H') and Pielou's Evenness (J) of the whole epifaunal assemblages and its individual components (molluscs, annelids and amphipods). If a non-linear relationship between response and depended variables was detected, we examined the significance of applying a quadratic term in the model. In addition, a distance-based redundancy analysis (dbRDA, Legendre and Anderson, 1999) was used to investigate the relationship between structural attributes and the epifaunal multivariate structure. Since dbRDA is susceptible to multicollinearity (i.e. high correlation between environmental variables), draftsman plots were used to verify skewness or identify clear correlations between structural attributes. A log(x + 1) transformation was used to correct the right-skewness of thallus volume (*TV*) and biomass (*DW*). Moreover, due to the high correlation between canopy volume (*CV*) and interstitial volume (*IV*) we removed *CV* from the subsequent analyses. Then, the structural attributes were normalised using a z-score transformation due to their varying measurement scales. Finally, forward selection was used to identify the structural properties that mostly contributed to the heterogeneity in the multivariate structure of the epifaunal assemblages.

Statistical analyses were performed using R open access statistical software 3.5.1 (R Core Team, 2018). See the "*Data availability and reproducible research*" section for further details.

Results

Epifauna

Overall, we identified 5676 individuals of epifauna belonging to 199 taxa (74 molluscs, 50 amphipods, and 75 annelids). Of these, 46 and 38 taxa were exclusively found on homogenous and mixed stands of the native *E. brachycarpa* respectively, while the invasive *A. taxiformis* hosted only 12 unique taxa (Fig. 2, Table S1-S2). Syllidae (annelids) and Rissoidae (molluscs) were the most species rich families (35 spp. and 21 spp. respectively), while all the other families contained less than 10 species each (Fig. 2, Table S1). Among molluscs, the most abundant species were *Eatonina cossurae* (Calcara, 1841) on both *E. brachycarpa* (186 ind.) and *A. taxiformis* (12 ind.) and *Setia ambigua* (Brugnone, 1873) on *E. brachycarpa* from mixed stands (161 ind.). The most abundant species among amphipods were *Ampithoe ramondi* on *E. brachycarpa* (164 ind.), *Apherusa alacris* (Krapp-Schickel, 1969) on *E. brachycarpa* from mixed stands (253 ind.) and *Caprella acanthifera* (Leach, 1814) on *A. taxiformis* (33 ind.). Finally, annelids were mostly represented by *Amphiglena mediterranea* (Leydig, 1851) on *E. brachycarpa* (457 ind.) and *Syllis prolifera* (Krohn, 1852) on both *E. brachycarpa* in mixed stands (171 ind.) and *A. taxiformis* (17 ind.) (Fig. 2, Table S1).

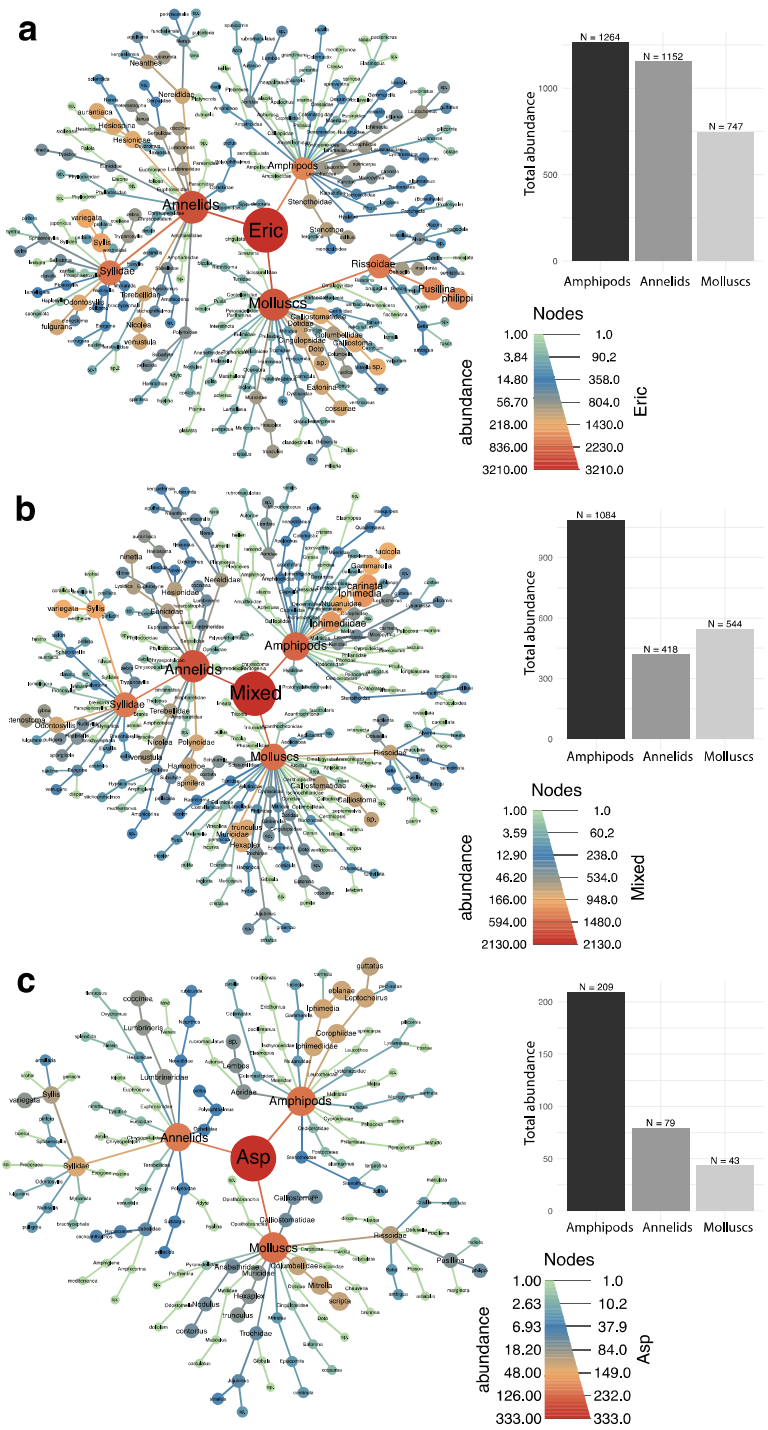

Fig. 2 Differences in the epifaunal assemblages among habitats. Heat trees show the abundances of taxa classified at the lowest taxonomic level possible on *E. brachycarpa* (a), *E. brachycarpa* in mixed stands (b) and *A. taxiformis* (c). Bar charts show the total abundance of amphipods, annelids, and molluscs for each habitat.

Total abundance (**N**) and rarefied species richness (**S**), differed significantly among habitats, with values that were higher in *E. brachycarpa* compared to *E. brachycarpa* in mixed stands and *A. taxiformis* (Fig. 3, Table S3). Shannon-Wiener diversity (H') varied significantly across habitats, with *E. brachycarpa* and *E. brachycarpa* in mixed stands exhibiting closer and higher values than *A. taxiformis.*

Conversely, Pielou's evenness (**J**) was higher in *A. taxiformis* compared to the other two habitats, which showed comparable values (Fig. 3, Table S3).

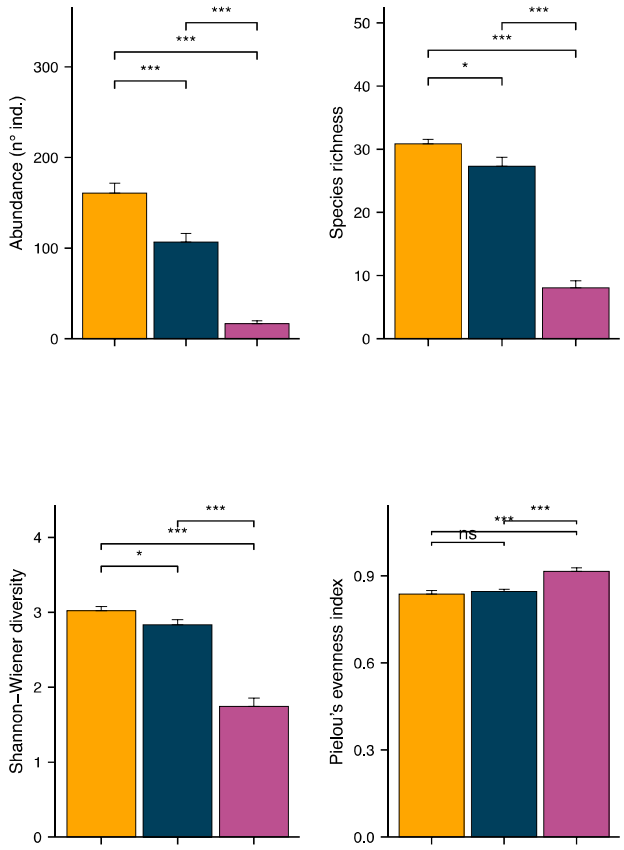

Fig. 3 Comparison of the alpha diversity indices among habitats. Abundance (a), rarefied species richness (b), Shannon-Wiener diversity (c), and Pielou's evenness index (d) of the epifaunal

assemblage associated with *E. brachycarpa*, *E. brachycarpa* in mixed stands, and *A. taxiformis*. Bar plots show mean +/- 1 standard error (n = 20). Significant codes: *** p<= 0.001, * p <= 0.05, ns p>0.05. See Table S3 for further details.

PERMANOVA showed that the structure and composition of the epifaunal assemblages differed significantly among habitats (Table S4). PERMDISP analysis revealed a high dispersion of samples within habitats, especially for *E. brachycarpa* in mixed stands and *A. taxiformis* (Fig. 4). Notwithstanding this high dispersion, the epifaunal assemblages of the three habitats were clearly separated, as shown by the PCoA ordination plot (Fig. 4). The proportion of variance explained by the first two axes was 62.8% for structure and 45.6% for composition. The first axis accounted for the larger part of the variance (structure = 49.5% and composition = 36.1%) and highlighted a shift, in both structure and composition, from *E. brachycarpa* to *A. taxiformis*, with *E. brachycarpa* in mixed stands placed between the two homogeneous stands of native and invasive seaweeds (Fig. 4). The second axis explained lower variation (structure = 13.3% and composition = 9.5%) and separated *E. brachycarpa* and *A. taxiformis* from *E. brachycarpa* in mixed stands (Fig. 4).

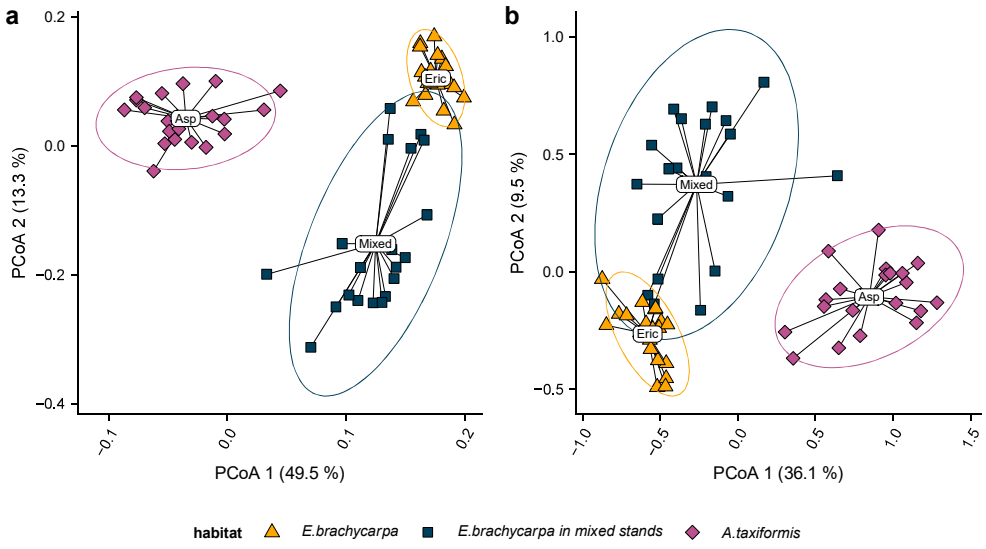

Fig. 4 Structure (a) and composition (b) of the epifaunal assemblages associated with the three habitats. The Principal coordinate analysis plot (PCoA) based on a Bray-Curtis distance matrix of square-root transformed relative abundances (structure) or on a Jaccard distance matrix of presence/absence data (composition). The circles show the 90% confidence interval for each seaweed.

The SIMPER analysis revealed that 28 taxa contributed 70% to the dissimilarity between *E. brachycarpa* and *A. taxiformis*; 37 taxa contributed 70% to the dissimilarity between *E. brachycarpa* and *E. brachycarpa* in mixed stands; and 30 taxa contributed 70% to the dissimilarity between *E. brachycarpa* in mixed stand and *A. taxiformis* (Fig. S1, Table S5). Most of the species contributing to the dissimilarities belonged to amphipods. The polychaete *Amphiglena mediterranea* (Leydig, 1851) was the species mostly contributing to the differences observed between both *E. brachycarpa* and *A. taxiformis* and between *E. brachycarpa* and *E. brachycarpa* in mixed stands, contributing respectively to 8% and 6% of the observed differences. The amphipod *Apherusa alacris* Krapp-Schickel, 1969 was the species most contributing to the differences (7%) between *E. brachycarpa* in mixed stands and *A. taxiformis*. In addition, the gastropod *Obtusella macilenta* (Monterosato, 1880) was the species that contributed consistently (higher δi/SD(δi) values) to the difference between *E. brachycarpa* and *A. taxiformis* (Fig. S1, Table S5), while the amphipod *Stenothoe monoculoides* (Montagu, 1813) and the gastropod *Eatonina cossurae* (Calcara, 1841) were the species that contributed consistently to the differences between *E. brachycarpa* in mixed stands and *E. brachycarpa*, and between *E. brachycarpa* in mixed stands and *A. taxiformis* (Fig. S1, Table S5). The polychaete *S. prolifera* was among the first 5 species contributing to the differences between each couple of habitats (Fig. S1, Table S5).

Multivariate analyses conducted separately for the three dominant epifaunal groups (molluscs, annelids, and amphipods) revealed patterns of variation comparable to those of the whole epifaunal assemblage (Table S6). Only, amphipods showed less variability among habitats (Table S6).

Seaweed structural attributes and relationships with the epifaunal assemblages.

Canopy volume (CV) and interstitial volume (IV) differed significantly among habitats with higher values in *A. taxiformis* compared to *E. brachycarpa* in mixed stands and *E. brachycarpa* (Fig. 5 a-b, Table S7). Biomass (DW) and thallus volume (TV) showed similar values between *E. brachycarpa* and *E. brachycarpa* in mixed stands and were significantly higher compared to those of *A. taxiformis* (Fig. 5 c-d, Table S7).

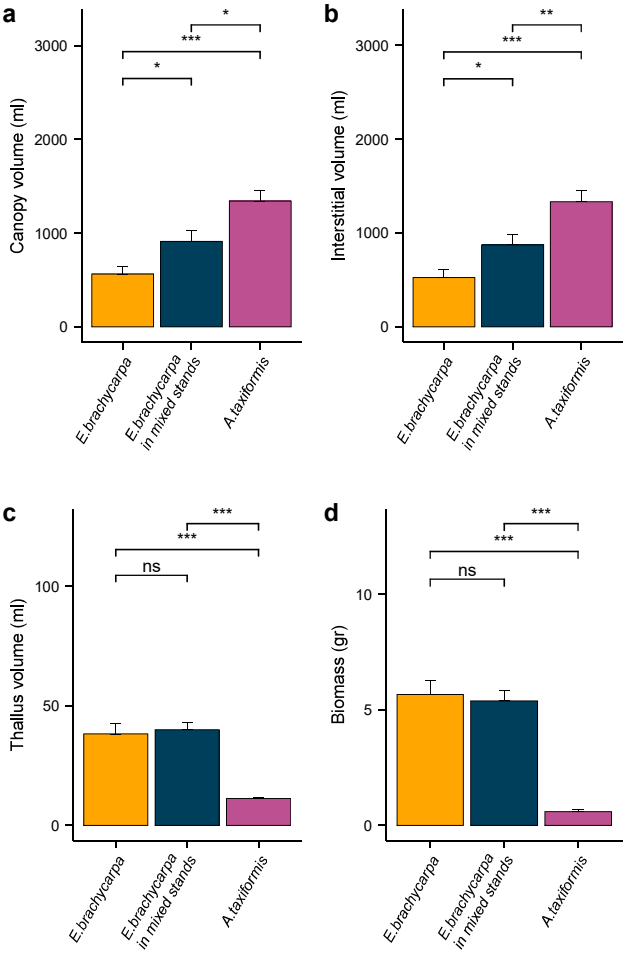

Fig. 5 Differences in structural attributes among habitats. Canopy volume (CV), interstitial volume (IV), thallus volume (TV) and biomass (expressed as dry weight, DW) of the epifaunal assemblages associated with *E. brachycarpa, E. brachycarpa* in mixed stands and *A. taxiformis*. Bar

plots show mean ± 1 standard error (n = 20). Significant codes: *** p<= 0.001, ** p <= 0.01, * p <= 0.05, ns p>0.05. See Table S7 for further details.

The linear regression analysis revealed that biomass (DW) was the attribute that explained better the variation in abundance ($R^2_N = 0.51$), rarefied species richness ($R^2_S = 0.58$), Shannon-Wiener diversity ($R^2_{H'} = 0.54$) and Pielou's evenness index ($R^2_j = 0.2$) of the epifaunal assemblages (Table S8). The variance explained by algal biomass increased if we considered a quadratic relationship between those variables (Fig. 6). The relationship was positive for N, S and H', while J presented and opposite pattern of variation (Fig. 6). Otherwise, canopy volume (CV) interstitial volume (IV) and thallus volume (TV) explained less variation (and it was significant for N, S and H' but not for J) of the epifaunal attributes (R-squared < 0.5, Table S8). The analysis conducted separately on the three dominant epifaunal groups (molluscs, annelids, and amphipods) revealed similar results however for amphipods and annelids the relationship between assemblage parameters and algal biomass was weaker (annelids: $R^2_N = 0.35$, $R^2_S = 0.52$, $R^2_{H'} = 0.49$, $R^2_J = 0.03$; amphipods: $R^2_N = 0.23$, $R^2_S = 0.43$, $R^2_{H'} = 0.31$, $R^2_J = 0.01$) although remaining the most important explanatory variable for both groups. Meanwhile, molluscs revealed patterns of variation similar to the whole assemblage ($R^2_N = 0.5$, $R^2_S = 0.53$, $R^2_{H'} = 0.48$, $R^2_J = 0.12$) (Table S8). As for the other algal structural features, annelids showed a weaker and not significant relationship with the canopy and interstitial volumes, differing from amphipods and molluscs (Table S8).

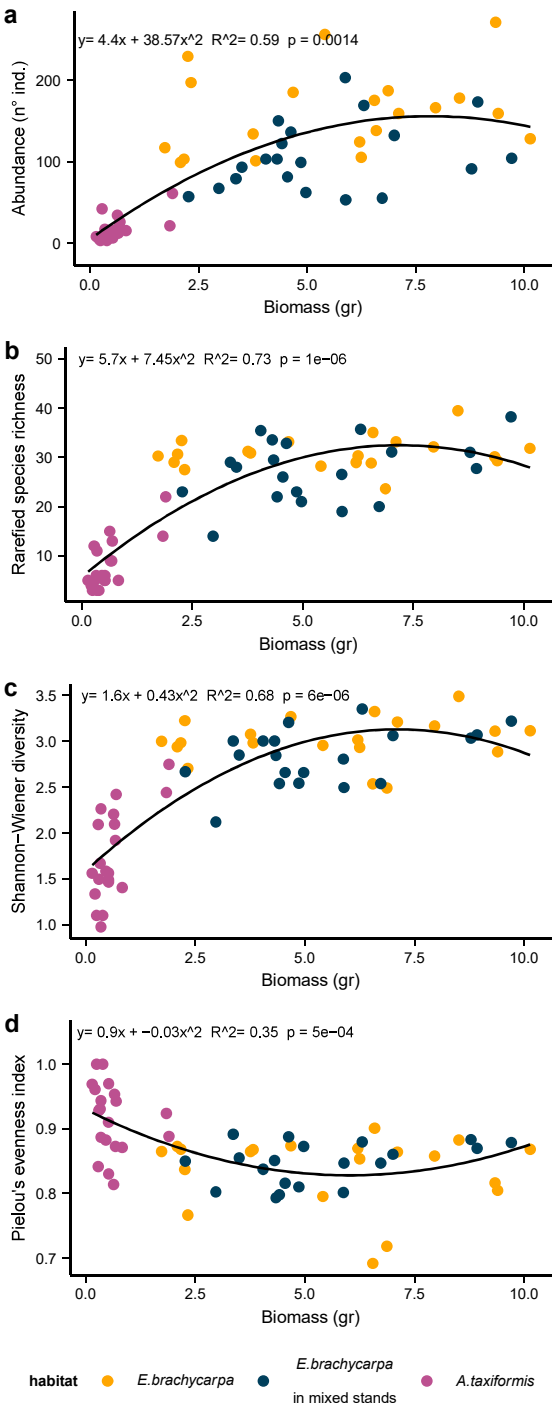

Fig. 6 Relationship between seaweed structural attributes and epifaunal diversity. Results of the linear regression analysis (LM) between the algal biomass (expressed as dry weight, DW) and the abundance (a), rarefied species richness (b), Shannon-Wiener diversity (c) and Pielou's evenness index (d) of the epifaunal assemblages.

Biomass (DW) was also the structural attribute selected for constrained db-RDA, explaining 24.7% of the variation in the structure of the epifaunal assemblages (Table S9). The first two axes of the dbRDA plot explained 15.6% of the total variance of the multivariate structure of the epifaunal assemblages, with 12.4% for axis 1 and 3.2% for axis 2 (Fig. 7).

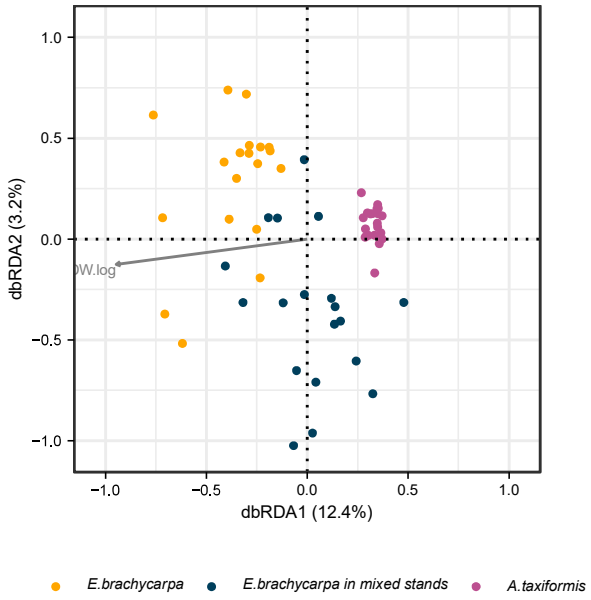

Fig. 7 Relationship between structural attributes and the multivariate structure of the epifaunal assemblages associated to the three habitats. The distance-based redundancy (dbRDA) plot illustrates the structural attribute better explaining the multivariate structure of the three habitats. DW.log = seaweed biomass (log + 1).

Discussion

The biodiversity and the socio-economic value of marine ecosystems are threatened by biological invasions around the world (Bax et al., 2003; Molnar et al., 2008). Understanding how invasive seaweeds modify the functioning of recipient ecosystems may allow us to better understand large scale effects on native rocky shore habitats. Here we investigated the effects of the invasive *A. taxiformis* on the native *E. brachycarpa* by comparing the epifaunal assemblage associated with three alternative states of the transition between native and invasive seaweeds, homogenous and mixed stands of the two seaweeds. Our results showed differences in abundance and diversity of the epifaunal assemblages between three alternative states of the transition from native *E. brachycarpa* to invasive *A. taxiformis.* In particular, *A. taxiformis* hosted almost 6 times less epifaunal individuals compared to *E. brachycarpa* in mixed stands, and 10 folds less individuals compared to homogenous stands of *E. brachycarpa*. Also, the number of epifaunal species was more than 4 folds lower in the invasive compared to the native habitat, while diversity reduced by half. These results confirm that generally invasive seaweeds exhibit a less diverse epifaunal component compared to native seaweeds (Guerra-García et al., 2012; Janiak and Whitlatch, 2012; Maggi et al., 2015; Navarro-Barranco et al., 2018; Veiga et al., 2018).

We found that variation in diversity and multivariate structure of the epifaunal assemblages was related to changes in algal structural features. In particular, biomass was the variable better explaining the variation in abundance, number of species, and the multivariate structure of the epifaunal assemblages. The role of macroalgal complexity in shaping the associated biota has been highlighted in several studies, with complex algae hosting a larger abundance and diversity of epifauna than simpler ones (Chemello and Milazzo, 2002; Pitacco et al., 2014; Veiga et al., 2014; Lolas et al., 2018; Veiga et al., 2018; Bitlis, 2019; Chiarore et al., 2019; Poursanidis et al., 2019; Mancuso et al., 2021b). In general, studies have highlighted that invasive seaweeds host lower (Guerra-García et al., 2012; Navarro-Barranco et al., 2018; Rubal et al., 2018; Veiga et al., 2018) or higher (Veiga et al., 2014) epifaunal abundance, species richness, and diversity, depending on whether their structural

complexity is respectively lower or higher compared to native seaweeds. Navarro-Barranco et al. (2018) showed that *A. taxiformis* had low fractal complexity and hosted an impoverished faunal assemblage compared to native seaweeds. Guerra-García et al., (2012) found that *A. armata* had low algal volume and showed lower abundance, species richness, and diversity of associated isopods fauna compared to the native *Ellisolandia elongata* (J.Ellis & Solander) K.R.Hind & G.W.Saunders. Moreover, lower dry weight and fractal dimension in the invasive *S. muticum* compared to native seaweeds have been shown to play a major role in shaping the associated faunal assemblages (Veiga et al., 2014, 2018). Likewise other studies (Janiak and Whitlatch, 2012; Veiga et al., 2014; Rubal et al., 2018), our results indicated that the quantity of habitat (biomass) was the best predictor variable explaining variation in terms of abundance, species richness, as well as multivariate structure of the associated epifauna.

Interestingly, our results highlight that *A. taxiformis* affected each component (molluscs, annelids, and amphipods) of the epifaunal assemblages in the same way. However, in the available literature on the epifaunal assemblages of invasive seaweeds, we can find distinct responses among epifaunal components (Schmidt and Scheibling, 2006; Gestoso et al., 2010; Guerra-García et al., 2012; Bedini et al., 2014; Veiga et al., 2018; Navarro-Barranco et al., 2019). For example, species richness, Shannon diversity and total abundance of isopods were significantly lower in *A. armata* compared to native algae (Guerra-García et al., 2012). Bedini et al. (2014) found that the invasive *Lophocladia lallemandii* (Montagne) F. Schmitz hosted a higher abundance of amphipods, isopods, and polychaetes, while native habitats harbored a greater abundance of molluscs and decapods. Bivalves associated with the invasive *S. muticum* were more abundant compared to native seaweeds, which, in contrast, hosted more gastropods (Veiga et al., 2018), and Gestoso et al. (2010) found that isopods and amphipods were more abundant in *S. muticum* than in native seaweeds. Moreover, the invasive *Codium fragile* subsp. *fragile* (Suringar) Hariot supported higher densities of nematodes, bivalves, and specialist herbivores compared to fronds of the native kelp, which, in contrast, supported greater densities of gastropods and asteroids (Schmidt and Scheibling, 2006). Other authors revealed that

differences between invasive and native seaweeds in single components of epifaunal assemblages changed depending on the site and the identity of the algal species (Navarro-Barranco et al., 2019). The fact that in our study, the *A. taxiformis* habitat showed lower abundance, species richness, and diversity values for all the epifaunal organisms, regardless of the groups investigated in this study (molluscs, amphipods and annelids), led us to hypothesize that a potential shift from the native (i.e. *E. brachycarpa*) to the invasive (i.e. *A. taxiformis*) habitat could cause large negative cascade effects on the benthic ecosystem.

Although differences in the epifaunal assemblages among native and invasive seaweeds have been largely explored, our results also suggest that the presence of *A. taxiformis* affects the epifaunal assemblages associated with *E. brachycarpa* in mixed stands. This result could be explained by other attributes that differed between native and invasive seaweeds, such as the amount of epiphytes and/or the presence of chemical defenses, that have been related to the ability of seaweeds to shape their associated fauna (Hay et al., 1987; Viejo, 1999; Paul et al., 2006; Cacabelos et al., 2010; Máximo et al., 2018; Gache et al., 2019). Invasive seaweeds can release secondary metabolites (e.g. halogenated compounds) able to act as deterrents against epiphytes, and herbivores (Paul et al., 2006; Cacabelos et al., 2010; Vega Fernández et al., 2019). Secondary metabolites released by *A. taxiformis* can affect the survival of fish in the post-larval stages, eventually leading to alteration of the grazing pressure on the surrounding habitat (Máximo et al., 2018; Gache et al., 2019). Other studies suggest that invasive seaweeds can alter the trophic web by changing the composition of epiphytes which reduces suitable habitat for many epifaunal species (Viejo, 1999; Wikström and Kautsky, 2004). Several authors suggested that the amount of epiphytes could explain the higher species richness found in the invasive *S. muticum* compared to native seaweeds (Viejo, 1999; Cacabelos et al., 2010). In our study, *A. taxiformis* had no or fewer epiphytes compared to *E. brachycarpa* (R.C. personal observation). As epifauna is mostly represented by microalgae grazers, we can hypothesize that differences in the abundance of epiphytes between *A. taxiformis* and *E. brachycarpa* could contribute to the variation in epifaunal assemblages observed in this study. It is therefore arguable that further studies analyzing

the direct and indirect role of epiphyte abundance and secondary metabolites released by *A. taxiformis* in structuring its associated epifauna would allow us to better clarify the effects of this seaweed on the recipient habitats.

Moreover, as suggested by other authors (Navarro-Barranco et al., 2019), landscape features could be another key aspect explaining the effect of *A. taxiformis* on *E. brachycarpa* associated assemblages in mixed stands. In fact, the presence of invasive seaweeds may contribute to the fragmentation of native habitats, reducing the patch size of native seaweeds, and at the same time increasing their isolation (Roberts and Poore, 2006; Lanham et al., 2015). It has been observed that the reduction in patch size of *Cystoseira sensu lato* habitats reduces the diversity of associated faunal assemblages (Mancuso et al., 2021b). Thus, we can hypothesize that the presence of A. taxiformis in mixed stands can act as a physical barrier to the dispersal of vagile fauna, reducing connectivity on a small scale and ultimately eroding the diversity of native habitats (Lanham et al., 2015). However, another possibility to consider is that some epifaunal groups may be able to disperse through the different seaweeds (Taylor, 1998) in mixed stands. In this case, the lower epifaunal abundance and diversity observed on *E. brachycarpa* from mixed stands could be because part of this diversity may have preferentially dispersed to the *A. taxiformis* portion of these mixed stands. Our study, however, cannot address the effects of epifauna movement among seaweeds on the observed results, and more studies are necessary to understand the possible role of mobile epifaunal assemblage movement patterns within mixed stands of *E. brachycarpa*.

One inherent weakness of this study concerns the impossibility of separating the effects of *A. taxiformis* from natural spatial variability. This should be accounted for by using more interspersed sites for each condition (mono and mixed stands of native and invasive seaweeds). Unfortunately, we were not able to find more interspersed sites in the area of study. However, the three sites selected had the same average values of different environmental variables (surface temperature, salinity, nitrate and phosphate concentrations, dissolved oxygen, chlorophyll and photosynthetic active radiation, Table S10), indicating that spatial variability had little effect. Then, we think that the data

presented in this study is still valuable, albeit with its limitations, for understanding epifaunal community changes between plausible alternative states of the transition between *E. brachycarpa* and *A. taxiformis*.

In summary, our study suggests that shifting from native to invasive habitats may pose a serious threat to biodiversity in coastal areas (Martin et al., 1992; Heck et al., 2003), potentially leading to bottom-up effects on rocky shore ecosystems. In addition, the low biomass supplied by the herein studied invasive species suggests that the shift from native canopy-forming algae to the invasive *A. taxiformis* habitat would also drastically reduce the biomass of primary producers in affected coastal areas. Predicting the ecological effects of invasive seaweeds is one of the main goals in the study of biological invasions. Previous research has highlighted the context-dependent effects of invasive seaweeds, with larger impact caused by invasive species exerting a different functional role compared to native habitat forming species (Ricciardi and Atkinson, 2004; Ricciardi et al., 2013; Navarro-Barranco et al., 2019). Our results not only remark the negative effect of *A. taxiformis* on *E. brachycarpa* epifaunal assemblages, but also suggest that invasive species are able to affect native habitats in a transitional phase (mixed stands) of the habitat shift, facilitating fragmentation and isolation. Further studies aimed at understanding the effects of the habitat shift from native to invasive seaweeds should include multiple transitional phases (different percentage coverage), as well as the analysis of changes in the trophic structure of the associated epifaunal assemblages.

Declarations

Not applicable

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Conflicts of interest/Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability and reproducible research

The repository with all the data and the scripts used to reproduce the research in this paper is available at http://dx.doi.org/10.17632/h3r8ygnjfd.1

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Author Contributions

Conceptualization, C.R., M.M. and D.R.; Methodology, C.R. and M.M.; Investigation, D.R.; Formal Analysis, M.F.P.; Data curation, M.F.P.; Writing – Original Draft Preparation, M.F.P.; Writing – Review & Editing, M.F.P., M.M., C.R., B.F., M.L., M.B., L.B.S.; Visualization, M.F.P.; Validation, M.F.P.; Supervision, C.R.; Project Administration, C.R.; Funding Acquisition, C.R.; Resources, C.R.; molluscs identification, C.R., D.R.; amphipods identification , L.B.S; annelids identification ; B.F., M.B., M.L.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

CRediT author statement

Conceptualization, C.R., M.M. and D.R.; Methodology, C.R. and M.M.; Investigation, D.R.; Formal Analysis, M.F.P.; Data curation, M.F.P.; Writing – Original Draft Preparation, M.F.P.; Writing – Review & Editing, M.F.P., M.M., C.R., B.F., M.L., M.B., L.B.S.; Visualization, M.F.P.; Validation, M.F.P.; Supervision, C.R.; Project Administration, C.R.; Funding Acquisition, C.R.; Resources, C.R.; molluscs identification, C.R., D.R.; amphipods identification , L.B.S; annelids identification ; B.F., M.B., M.L.

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