


The role of competition and herbivory in biotic resistance against invaders: a synergistic effect

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Abstract. Invasive species pose a major threat to global diversity, and once they are well established their eradication typically becomes unfeasible. However, certain natural mechanisms can increase the resistance of native communities to invaders and can be used to guide effective management policies. Both competition and herbivory have been identified as potential biotic resistance mechanisms that can limit plant invasiveness, but it is still under debate to what extent they might be effective against well-established invaders. Surprisingly, whereas biotic mechanisms are known to interact strongly, most studies to date have examined single biotic mechanisms separately, which likely influences our understanding of the strength and effectiveness of biotic resistance against invaders. Here we use long-term field data, benthic assemblage sampling, and exclusion experiments to assess the effect of native assemblage complexity and herbivory on the invasion dynamics of a successful invasive species, the alga *Caulerpa cylindracea*. A higher complexity of the native algal assemblage limited *C. cylindracea* invasion, probably through competition by canopy-forming and erect algae. Additionally, high herbivory pressure by the fish *Sarpa salpa* reduced *C. cylindracea* abundance by more than four times. However, long-term data of the invasion reflects that biotic resistance strength can vary across the invasion process and it is only where high assemblage complexity is concomitant with high herbivory pressure, that the most significant limitation is observed (synergistic effect). Overall, the findings reported in this study highlight that neglecting the interactions between biotic mechanisms during invasive processes and restricting the studied time scales may lead to underestimations of the true capacity of native assemblages to develop resistance to invaders.

Key words: alga–herbivore interactions; biological invasions; biotic resistance; *Caulerpa cylindracea*; herbivory; interspecific competition; invasion ecology; *Sarpa salpa*.

INTRODUCTION

Biological invasions are one of the main threats to biodiversity and ecosystem function worldwide, being the second most prominent cause of species extinctions and playing an important role in diversity reduction (Vilà et al. 2011, Bellard et al. 2016). Furthermore, bioinvasions can produce alterations in a number of ecosystem services and basic ecosystems processes (Pejchar and Mooney 2009, Vilà et al. 2010, Simberloff et al. 2013), often at great economic cost (Pimentel et al. 2005). Still, our understanding of the factors that

influence invasion success remains limited (Simberloff et al. 2013), complicating the development of effective management strategies to prevent and mitigate the negative effects of invasive species.

The success of an invasion is dependent on multiple processes across a wide range of temporal and spatial scales (Perelman et al. 2007, Theoharides and Dukes 2007, Eschtruth and Battles 2009a, Byun et al. 2015). Among these processes, most of the attention has fallen on biological processes, in the context of the Biotic Resistance Hypothesis (Elton 1958, Keane and Crawley 2002, Levine et al. 2004). The strength of biotic resistance against an invader is strongly influenced by the native assemblage and by the functional traits of the native species (Pokorny et al. 2005, Perelman et al. 2007, Byun et al. 2013), which modulate the interspecific

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competition; but also by the consumer pressure on both the invasive and the native species (Levine et al. 2004, Parker and Hay 2005, Mitchell et al. 2006). In this sense, negative effects of competition on several invasive plants, mainly caused by the limitation of essential resources such as water, nutrients or light, have been previously reported in tropical forests (Fine 2002), salt-marshes (Amsberry et al. 2000), grasslands (Corbin and D'Antonio 2004, te Beest et al. 2018), mangroves (Li et al. 2014, Zhang et al. 2018), shrublands (Morris et al. 2015), and freshwater ecosystems (Petrizzella et al. 2018). However, competition alone might not be enough to exert a strong biotic control against a well-established invader (Levine et al. 2004, Vilà and Weiner 2004). On the other hand, herbivory has been also acknowledged as an important biotic resistance mechanism for native ecosystems (Levine et al. 2004, Parker and Hay 2005, Parker et al. 2006), although its effectiveness is controversial (Maron and Vilà 2001, Keane and Crawley 2002, Liu and Stiling 2006). In fact, herbivores can promote (Eschtruth and Battles 2009b, Relva et al. 2010, Kalisz et al. 2014), deter (Cushman et al. 2011, Pearson et al. 2012, Zhang et al. 2018), or have no effect on the dominance of invasive plant species (Stohlgren et al. 1999), which makes it difficult to understand the conditions under which herbivory can be an effective biotic resistance mechanism against an invasion. Taking into account that herbivory can be highly influenced by other factors such as native plant traits (Grutters et al. 2017) or habitat features (Alofs and Jackson 2014, Li et al. 2014, Ender et al. 2017, Zhang et al. 2018), contrasting observations on the role of biotic mechanisms in controlling invasive species may be partially explained by the fact that they are often assessed neglecting the relative importance of the interactions between mechanisms (Levine et al. 2004, Mitchell et al. 2006, Alofs and Jackson 2014, Zhang et al. 2018, Petrizzella et al. 2020). Indeed, the interaction between biotic mechanisms has been suggested to be responsible for an enhancement in the biotic resistance capacity of the invaded community (Suwa and Louda 2012, Li et al. 2014, Zhang et al. 2018).

In marine ecosystems, macroalgae are one of the most conspicuous and successful invaders, as well as one of the most harmful, comprising 20% of marine invasive species worldwide (Schaffelke et al. 2006) and causing potentially important ecological and economic damage (Williams and Smith 2007). Despite this, the factors that drive their invasive success remain largely unknown (Inderjit et al. 2006), although similarly to what has been observed for plant invasions in terrestrial ecosystems, both competition and herbivory are suspected to determine their invasion success (Kimbrow et al. 2013, Papacostas et al. 2017). In this regard, functional traits of the native species can influence habitat resistance to algae invasion through their contribution to interspecific competition (Arenas et al. 2006, Britton-Simmons 2006, Vaz-Pinto et al. 2012).

Surprisingly, however, most studies seem to confirm the inability of herbivores to limit the spread of well-established invasive macroalgae (e.g., Wikström et al. 2006, Forslund et al. 2010, Cebrian et al. 2011, Tomas et al. 2011b, Nejrup et al. 2012), despite the important role of herbivory regulating algal abundance and distribution (Vergés et al. 2009, Poore et al. 2012). Overall, previous research on biotic resistance against invasive macroalgae seems to suggest that, in most cases, the effect of single biotic mechanisms might not be enough to significantly affect invader performance (Kimbrow et al. 2013, Papacostas et al. 2017). Probably, as has been suggested above, only by considering the interaction between biotic mechanisms (both competition and herbivory), can more robust conclusions on the true resistance of an assemblage towards a particular invader be obtained.

In this study, we aim to test whether herbivory interacts with competition to modulate the resistance of a marine habitat towards a particular invader. We use *Caulerpa cylindracea*, one of the most invasive macroalgae worldwide, to assess the simultaneous role that both assemblage structure and herbivory pressure have on *C. cylindracea* invasion by using in situ field experiments that assess herbivory and assemblage characteristics. Additionally, we monitor the abundance of the invader over time to further elucidate whether the studied biotic mechanisms and their interaction influence the long-term dynamics of the invader.

MATERIALS AND METHODS

Study species

Caulerpa cylindracea is a green alga, native to the Southwestern coast of Australia (Verlaque et al. 2003), which is currently considered one of the most invasive species within the Mediterranean Sea (Klein and Verlaque 2008, Katsanevakis et al. 2016), having also invaded areas in the North Atlantic (Verlaque et al. 2004). Actually, it has recently been ranked as one of the marine invaders with the highest negative ecological impacts worldwide (Anton et al. 2019). However, despite its formidable ability to spread and grow, the abundance of *C. cylindracea* appears to differ markedly among invaded assemblages (Klein and Verlaque 2008, Cebrian and Ballesteros 2009), suggesting that there might be, in some cases, some natural mechanisms controlling *C. cylindracea* abundance. Among such mechanisms, competition may play an important role, because canopy-forming and erect algae (typical of high-complexity assemblages) can outcompete *C. cylindracea* (Ceccherelli et al. 2002, Bulleri and Benedetti-Cecchi 2008, Piazzini and Balata 2009, Bulleri et al. 2010), whereas herbivory seems to fail as a control mechanism for *C. cylindracea* when it is well established (Bulleri et al. 2009, Cebrian et al. 2011), even though several species are known to feed on it commonly (Ruitton et al. 2006, Box et al. 2009,

Cebrian et al. 2011, Tomas et al. 2011b). However, it is important to consider that previous studies have focused on only one of these mechanisms (either competition or herbivory) while, in nature, both mechanisms might act together to influence the abundance of the invasive species.

Study system

The Mediterranean Sea is the largest and deepest semienclosed sea on Earth and it is considered a hotspot for marine biodiversity as it harbors around 17,000 marine species, 20% of them being endemic to the region (Coll et al. 2010). Because of its temperate climatic conditions, Mediterranean benthic shallow habitats are dominated by macroalgae. Unfortunately, the Mediterranean Sea is one of the areas most susceptible to the introduction of nonnative species worldwide (Galil 2007) and it is considered to be a hotspot for invasive algae (Williams and Smith 2007, Thomsen et al. 2016).

The study was conducted in the Cabrera Archipelago National Park, in the Balearic Islands (western Mediterranean; 39°12'21" N, 2°58'44" E; Fig. 1). This maritime-terrestrial national park was established in 1991 and currently harbors some of the best-preserved benthic and fish assemblages in the Mediterranean Sea (Sala et al. 2012, Guidetti et al. 2014).

The invasive alga, *C. cylindracea*, was first detected in the area in 2003 at a depth of 30 m (Cebrian and Ballesteros 2009) and has, since then, colonized most of the park's benthic habitats at depths from 0 to 65 m. Indeed, in some of these habitats, it has become the dominant species (Cebrian et al. 2011).

Benthic habitat sampling and assemblage complexity

To assess the role that benthic assemblage complexity might have on *C. cylindracea* coverage, different assemblages were surveyed in three sites around the Cabrera Archipelago: Ses Rates, Na Foradada, and Freu de la Imperial (Fig. 1). A shallow assemblage (10 m) and a deep assemblage (30 m) were surveyed at each site to take into account the wide range of benthic assemblage complexities (Ballesteros et al. 1993) and contrasting herbivory pressures (Vergés et al. 2009, Tomas et al. 2011b) in relation to depth. Assemblages were sampled in 2005, 2006, and 2007. At each site and depth, three random samples measuring 20 × 20 cm² were collected, with the whole benthic cover removed using a hammer and a chisel (Boudouresque 1971, Sant et al. 2017). After removing the erect algae, and before scraping each quadrat, the cover of each encrusting species was visually estimated to obtain a more reliable value of their abundance in the encrusting layer. After collection, samples were preserved in 4% formalin in seawater, and once in the laboratory, they were sorted and all algae were identified to species level. Species coverage was calculated by placing the species specimens horizontally over a laboratory

tray and measuring the area they covered (Ballesteros 1986). Then, each algal species was assigned to a different category ("Canopy-forming," "Erect," "Turf," and "Encrusting") based on their morphological traits (size and morphology; Appendix S1: Table S1). In order to avoid the effect of assemblage complexity being subject to a confounding effect of the presence of *C. cylindracea*, this species was not included in any of the previous categories. Finally, the percent cover of each category in the sample was calculated.

Capacity of canopy-forming and erect algae to out-compete *C. cylindracea* in contrast to turf and encrusting species has been experimentally proven for coastal shallow rocky bottoms of the Mediterranean Sea (Ceccherelli et al. 2002, Bulleri and Benedetti-Cecchi 2008, Piazzini and Balata 2009, Bulleri et al. 2010). Consequently, complexity of each sample was defined based on the percentage abundance of the "Canopy-forming" and "Erect" categories. Three levels of complexity were defined for the samples based on the percentage of coverage that comprised canopy-forming and/or erect species: "high complexity" (more than 50%); "medium complexity" (between 15 and 50%), and "low complexity" (lower than 15%).

Exclusion experiment

An exclusion experiment was performed to assess whether fish herbivory could act as a biotic resistance mechanism against *C. cylindracea* invasion by reducing the abundance of the invasive alga. To this end, in order to obtain a proxy of contrasting herbivory intensities, and bearing in mind that herbivory pressure decreases strongly through the water column (Vergés et al. 2009, 2012, Steneck et al. 2017), the exclusion experiment was performed at two different depths: 10 m, where herbivory pressure is high, and 30 m, where it is low (Reñones et al. 1997, Tomas et al. 2011b, Vergés et al. 2012).

This experiment mainly targeted *Sarpa salpa*, because it is the only truly herbivorous fish in the western Mediterranean Sea (Verlaque 1990, Gianni et al. 2017), it plays an important role structuring algal communities (Vergés et al. 2009) and it regularly consumes *C. cylindracea* (Ruitton et al. 2006, Tomas et al. 2011b). This fish species is quite abundant throughout the Cabrera Archipelago, where it can reach densities up to 14 individuals per 250 m² above depths of 20 m (Reñones et al. 1997), spending around 90% of the time above 20 m (Tomas et al. 2011b). *Sarpa salpa* is known to have a quite heterogeneous diet (Havelange et al. 1997), but it can also be very selective (Verlaque 1990, Marco-Méndez et al. 2017) and even shows a preference for *C. cylindracea* over many native species (Tomas et al. 2011b), which makes it a potential candidate for the control of *C. cylindracea*.

The exclusion experiment was set up at the end of June 2011 in Na Foradada (Fig. 1), an area where fish

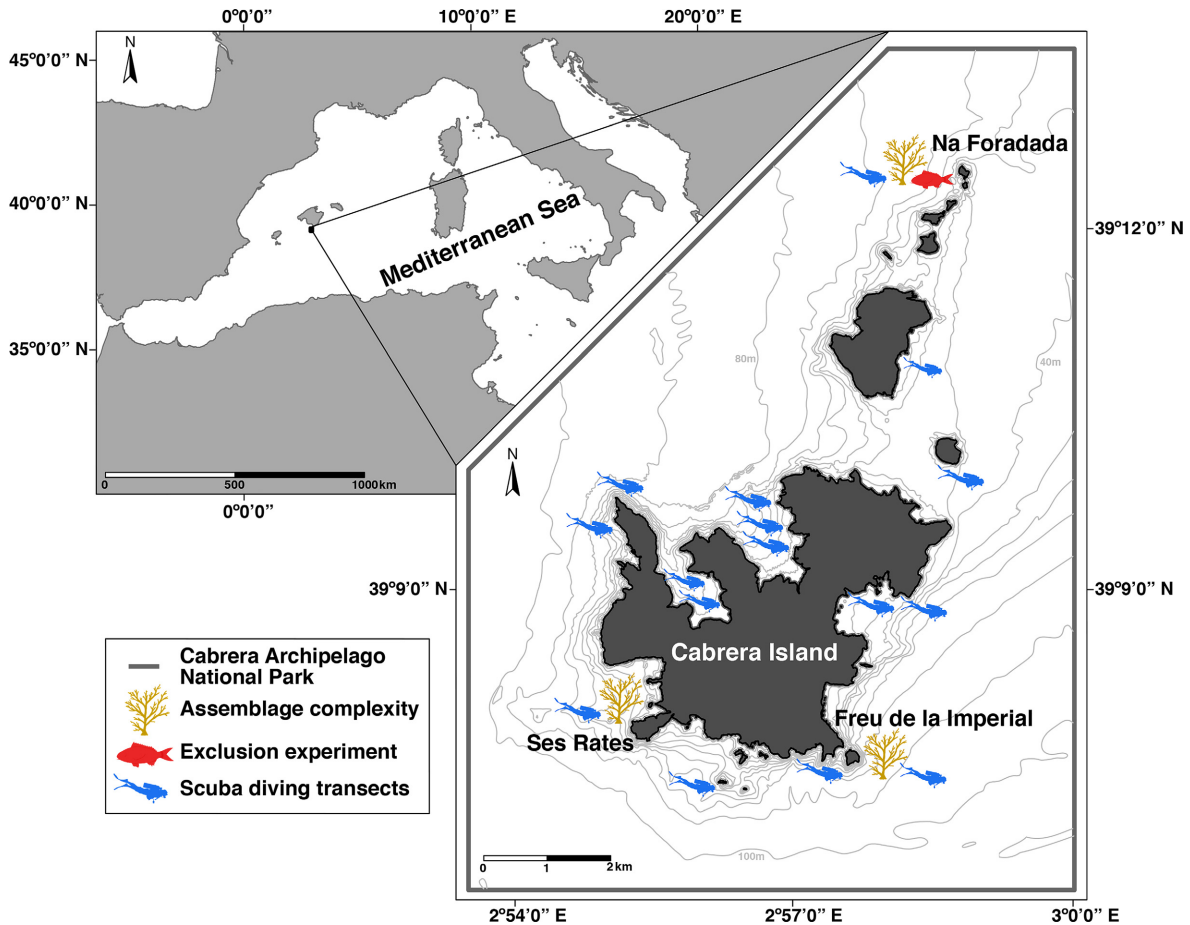


FIG. 1. Location of the Cabrera Archipelago National Park. The points show the locations of the sampling sites where assemblage complexity was assessed (3 sites), the exclusion experiment was performed (1 site) and the scuba diving transects were done (16 sites). Shapefiles for the Mediterranean Sea www.naturalearthdata.com and for the Cabrera Archipelago National Park www.miteco.gob.es.

communities are well established, sea urchin (*Paracentrotus lividus* and *Arbacia lixula*) densities are very low ($<0.1/\text{m}^2$) (Cebrian et al. 2011) and the highest densities of the fish *S. salpa* are found within the National Park, with more than 20 individuals per 250 m^2 (Reñones et al. 1997). Furthermore, we chose this area because both the shallow and the deep benthic habitats displayed a similar medium complexity (with coverage of erect and canopy-forming species at between 25% and 35%), and similar abundances of native species. This meant there was sufficient abundance of *C. cylindracea* to assess, in the field, the effect of herbivory pressure on it.

At each depth, three treatments were used: “Exclusion,” which consisted of cages of $50 \times 50 \times 50\text{ cm}^3$ made of plastic netting with a mesh size of 2.5 cm; “Control-Exclusion,” consisting of cages with open sides; and “Control,” consisting of $50 \times 50\text{ cm}^2$ quadrats marked permanently on the corners and without a cage. A total of five interspersed replicates per treatment were set (15 plots per depth) within an area of $<100\text{ m}^2$ to

avoid different abiotic conditions between plots. At the beginning (July) and at the end (August) of the experiment, pictures were taken at each plot to assess *C. cylindracea* abundance subsequently, which was calculated with the computer program *photoQuad* version 1.4 (Trygonis and Sini 2012). In each photograph, 50 random points were placed and then, each of these points was assigned to the category of either “*C. cylindracea*” or “other algae.” The proportion of points in each category was then used as a proxy of the percentage abundance for each of those two categories.

Abundance of *Caulerpa cylindracea*

The abundance of *C. cylindracea* at the Cabrera Archipelago was assessed in 2008 and then again in 2017. To do so, 16 representative sites around the archipelago were chosen (Fig. 1) and a perpendicular transect to shore was performed at each site by means of scuba diving. The depth of the transects ranged from 5 to 45 m to

cover the main bathymetric range at which *C. cylindracea* can be found (Cebrian et al. 2011). The abundance of *C. cylindracea* was estimated by means of $25 \times 25 \text{ cm}^2$ quadrats, divided into 25 subquadrats of $5 \times 5 \text{ cm}^2$ (Sala and Ballesteros 1997, Sant et al. 2017) and the number of subquadrats where *Caulerpa* was detected, was used as a unit of abundance. A total of 30 quadrats were randomly positioned within each 5-m-depth range, and then the mean *C. cylindracea* abundance per each 5-m-depth stratum was calculated. Also, to take into account the effect that assemblage complexity might have on the bathymetric abundance of *C. cylindracea*, each 5-m-depth range at each site was classified as having either high or low complexity based on the dominant morphological categories in the assemblage (“canopy-forming,” “erect,” “turf,” and “encrusting”), while ignoring the presence of *C. cylindracea* to avoid confounding effects.

Statistical analysis

The effect of benthic assemblage complexity on the cover of *C. cylindracea* was assessed with binomial generalized linear models (GLM), because the response variable was measured as a percentage (percentage of *C. cylindracea* coverage in each sample) and it could be approximated to a logistic distribution (e.g., success = % coverage of *C. cylindracea*, failure = % coverage of species other than *C. cylindracea*). Two models were fitted, one to assess the role of “assemblage complexity” and another to assess the role of both “depth” and “assemblage complexity” on *C. cylindracea* coverage. In the latter, both factors were included as fixed effects, and if the interaction between them was significant, it was also included in the model. To fit both models, the statistical environment R was used (R version 3.6.3) (R Development Core Team 2018); and to compare the effects between levels in the assemblage complexity factor itself (“high complexity,” “medium complexity,” and “low complexity”), and in the assemblage complexity factor at each depth (“shallow” and “deep”), Tukey post hoc tests were performed using the functions “pairs” and “emmeans” from the package *emmeans* (Lenth 2018).

To evaluate the effect of the exclusion treatment on the abundance of *C. cylindracea* at the end of the experiment, binomial mixed-effects GLMs were used because the response variables were measured as proportions and could be approximated to a logistic distribution (e.g., success = points that corresponded to *C. cylindracea*; failure = points that did not correspond to *C. cylindracea*), and the random terms were used to take into account the repeated measures. In the models, the factors “treatment” and “time” were included as fixed effects, whereas “plot” was included as a random effect. If the interaction between “treatment” and “time” was significant, it was also included in the fixed part of the model. Two models were fitted, one per depth (10 or 30 m) by means of the package *lme4* (Bates et al. 2015) for

R. Tukey post hoc tests, which were performed using the functions “pairs” and “emmeans” in the package *emmeans*, were used to compare effects between levels in the treatment factor (“Exclusion,” “Control-Exclusion,” “Control”) at each time observation (“beginning” and “end”).

The R code used to perform all the statistical analyses can be found on Zenodo⁶ (Santamaria 2021).

RESULTS

Effect of benthic assemblage complexity on *Caulerpa cylindracea* coverage

Benthic assemblage complexity had a significant effect on *C. cylindracea* coverage ($P < 0.001$, Appendix S2: Table S1), with high complexity assemblages supporting low *C. cylindracea* covers (Fig. 2A). In fact, there were significant differences in *C. cylindracea* cover among the different levels of assemblage complexity ($P < 0.001$, Appendix S2: Table S2), with *C. cylindracea* coverage lower than 5% in very complex assemblages, whereas in low-complexity assemblages, coverage of the invasive species reached values of almost 30% (Fig. 2A).

When the depth of the sampled assemblages was included in the model, *C. cylindracea* coverage varied significantly by depth, by assemblage complexity and by the interaction term (Appendix S2: Table S3). In fact, although the previous pattern of lower *C. cylindracea* cover in high complexity assemblages is maintained, the coverage of the invasive alga is significantly higher at deeper habitats, independently of assemblage complexity (Appendix S2: Table S4, upper table; Fig. 2B). In particular, in shallow habitats, high-complexity assemblages had 20 times less *C. cylindracea* coverage than low complexity assemblages, but only 10 times less coverage at deeper habitats (Fig. 2B). At each depth, there were significant differences between all levels of assemblage complexity, except between high complexity and medium complexity assemblages in shallow areas (Appendix S2: Table S4, lower table).

Exclusion experiment

Significant effects of herbivory on *C. cylindracea* abundance were only detected at shallow depths (10 m), where *C. cylindracea* abundance varied significantly by treatment, by time and by the interaction between the two (Appendix S3: Table S1). Although at the beginning of the experiment, all treatment areas displayed similar *C. cylindracea* abundance, both the “Control-Exclusion” and the “Control” treatments exhibited lower *C. cylindracea* abundances at the end of the experiment ($P < 0.0001$; Appendix S3: Table S2, upper table), whereas *C. cylindracea* abundance in the “Exclusion” treatment remained constant (Appendix S3: Table S2, lower table).

⁶ <https://doi.org/10.5281/zenodo.4664432>

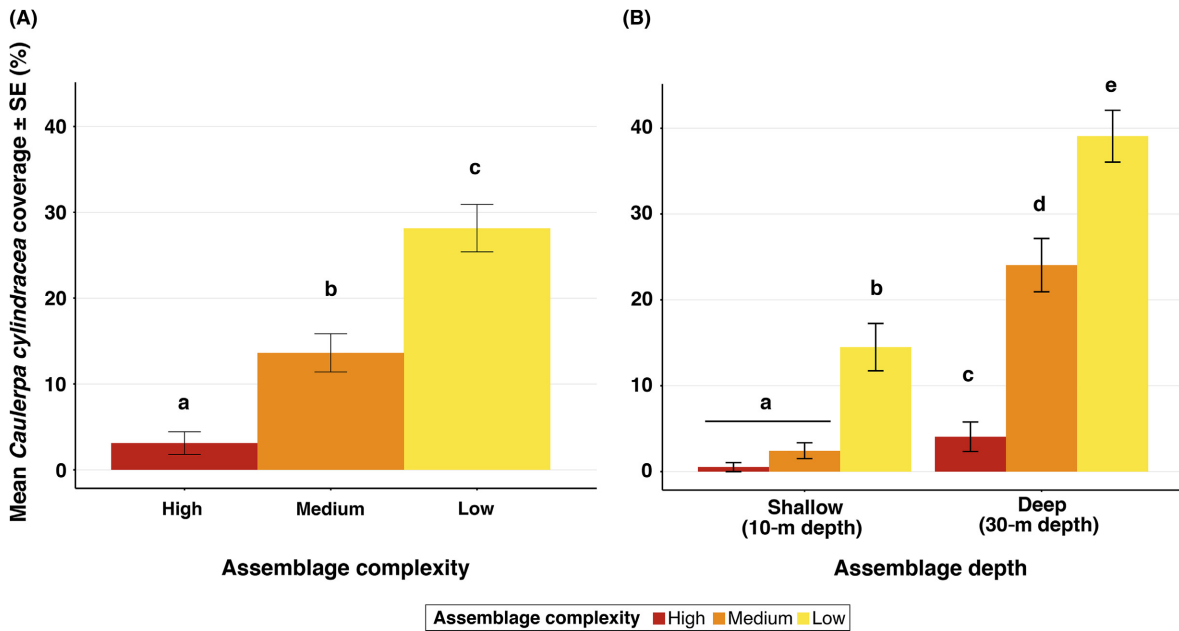


FIG. 2. Mean *Caulerpa cylindracea* coverage \pm SE (A) for each level of assemblage complexity, and (B) for each level of assemblage complexity at the two different depths. Significant differences between assemblage complexity levels (P values from Tukey's test with 95% confidence intervals) are indicated with letters.

Indeed, at the end of the experiment, *C. cylindracea* abundance was 4.33 and 2.36 times higher in the full exclusion cages compared with the uncaged control plots and the side-open cages, respectively (Fig. 3A).

On the other hand, at 30 m, the abundance of *C. cylindracea* varied significantly across time but not between treatments (Appendix S3: Table S1). In fact, for all three treatment levels, abundance was significantly higher (1.88 times on average) at the end of the experiment, than at the beginning (Fig. 3B).

Abundance of *Caulerpa cylindracea*

The abundance of *C. cylindracea* in the Cabrera Archipelago decreased between 2008 and 2017 at depths of between 5 and 35 m, but remained more or less constant below 40 m (Fig. 4). In the entire bathymetric distribution, assemblage complexity showed a considerable effect on *C. cylindracea* abundance, with high-complexity assemblages exhibiting lower abundances of the invasive alga than low complexity assemblages. Furthermore, it was in shallow and highly complex assemblages, from 5 to 25 m deep, where *C. cylindracea* reached its lowest abundance, with values ranging between 5 and 10% (Fig. 4B).

DISCUSSION

Our results indicate that although both competition and herbivory can provide biotic resistance to a certain extent, strong synergistic effects are observed when the

two mechanisms act together. Indeed, competition (by canopy-forming and erect algae) and herbivory (by *Sarpa salpa*), significantly affect the invasion outcomes of *C. cylindracea*, particularly limiting its abundance in complex algal assemblages in which herbivory pressure is high.

Benthic assemblage complexity had a strong influence on preventing *C. cylindracea* invasion. The dominance of canopy-forming and erect species resulted in reduced cover of *C. cylindracea*, probably determining resistance to *C. cylindracea* invasion through competition mechanisms (Ceccherelli et al. 2002, Piazzini and Balata 2009), such as the reduction in light availability, which can limit the photosynthetic performance of *C. cylindracea* (Bernardeau-Esteller et al. 2015, 2020, Marín-Guirao et al. 2015) and the prevention of reattachment of fragments through the branch-sweeping of the substratum (Bulleri and Benedetti-Cecchi 2008, Piazzini et al. 2016). In contrast, when the abundance of canopy-forming algae was low and the communities were dominated by turf and encrusting species, *C. cylindracea* cover was much higher. In such habitats, the colonization and spread of *C. cylindracea* seems to be facilitated because turf assemblages provide an optimal 3-D matrix that favors the anchoring of the stolons and trapping of fragments (Ceccherelli et al. 2002, Bulleri and Benedetti-Cecchi 2008, Bulleri et al. 2009). In this sense, these findings support previous evidence from marine and terrestrial ecosystems, where the presence and dominance of species with certain functional traits (e.g. growth form, size and height) among the assemblage, strongly

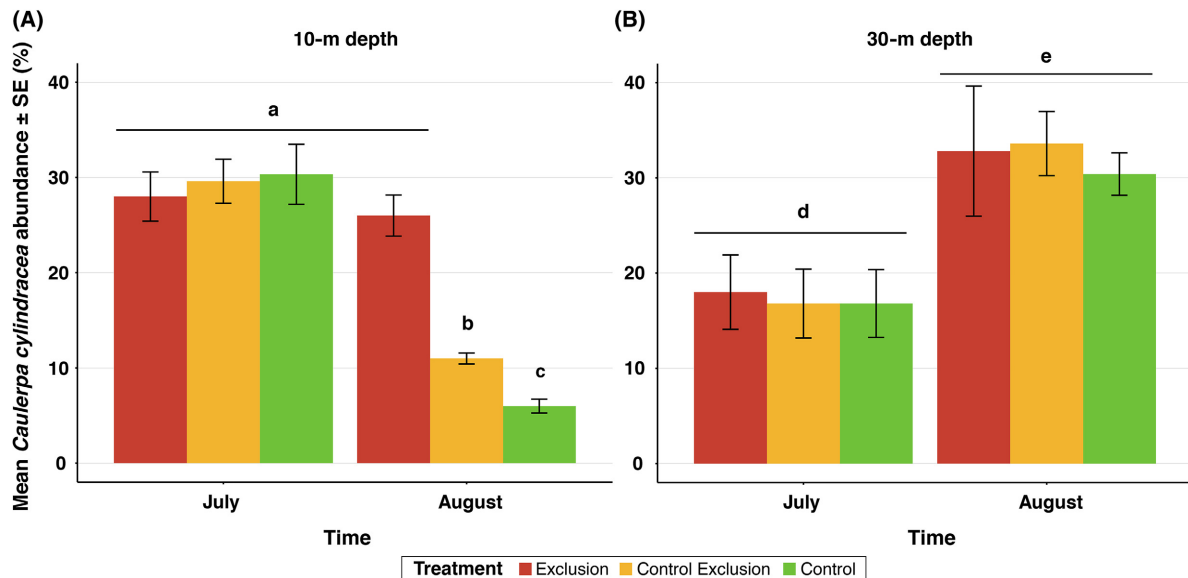


FIG. 3. Mean *Caulerpa cylindracea* abundance \pm SE, at each time observation and for each treatment in the exclusion experiment. (A) Exclusion experiment at 10-m depth and (B) exclusion experiment at 30-m depth. Significant differences between exclusion treatments (P -values from Tukey's test with 95% confidence intervals) are indicated with letters in each graph.

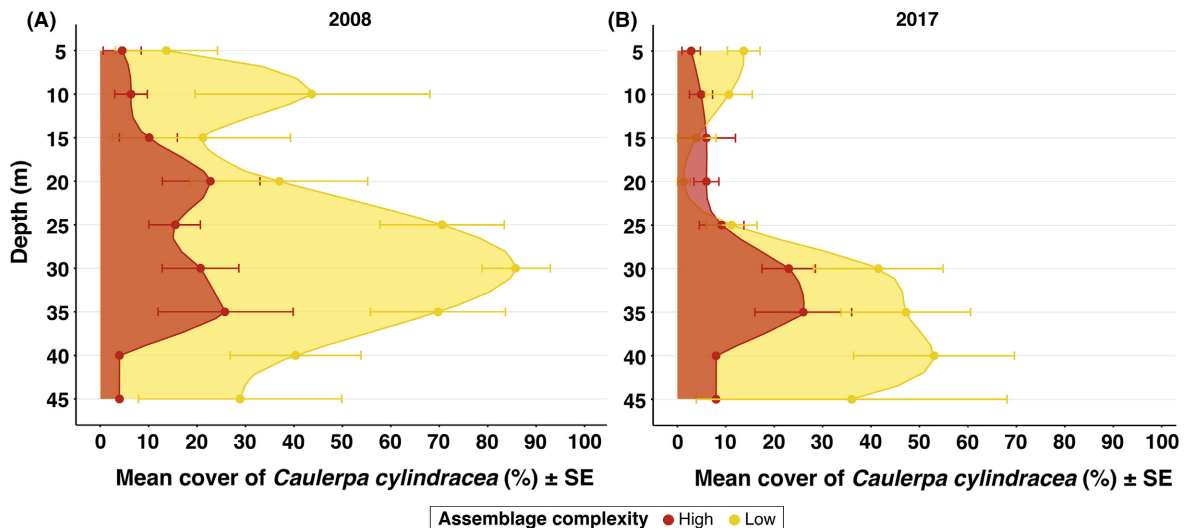


FIG. 4. Bathymetric abundance of *Caulerpa cylindracea* (mean \pm SE) at the Cabrera Archipelago National Park: (A) in 2008 and (B) in 2017, on assemblages with different complexities.

influences the strength of the biotic resistance against invasive primary producers (Lindig-Cisneros and Zedler 2002, Arenas et al. 2006, Britton-Simmons 2006, Byun et al. 2013, Bernardeau-Esteller et al. 2020) by limiting one or several essential resources for the invader.

However, the significant differences we observed in the abundance of *C. cylindracea* between shallow and deep communities, regardless of assemblage complexity, suggest that mechanisms other than assemblage

complexity are playing an important role on the invasion of *C. cylindracea*, particularly given that *C. cylindracea* can readily colonize habitats from 0 to 50 m depth (Klein and Verlaque 2008, Cebrian and Ballesteros 2009). In this sense, although several factors (e.g., propagule pressure, disturbance, or abiotic conditions) cannot be disregarded, we suggest that the observed differences in invader abundance mainly reflect the contrasting consumer pressures found between deep and shallow

communities. Concretely, given similar assemblage complexity, when herbivory pressure was high (here shallow habitats) (Reñones et al. 1997, Vergés et al. 2009, 2012, Tomas et al. 2011b), the abundance of *C. cylindracea* was significantly lower compared to areas where herbivory pressure was low or nonexistent (deeper habitats). Actually, both the exclusion experiment and the pattern of *C. cylindracea* abundance and distribution across the Cabrera Archipelago further support that herbivory is also contributing to the biotic resistance of native assemblages to the *C. cylindracea* invasion. In this sense, where herbivory is weak, such as in deep habitats, or if herbivores have been depleted, *C. cylindracea* is subject to limited control and its abundance depends largely on assemblage complexity, which results in higher abundances of the invader among the native assemblage (Fig. A, B). Similarly, when herbivory pressure is high but assemblage complexity is low, the invader will also suffer limited control (Fig. 5C). However, when high herbivory pressure is concomitant with high assemblage complexity, both mechanisms (competition and herbivory) strongly limit the abundance of *C. cylindracea* (Fig. 5D). Taking into account that sea urchin densities were very low in the study area (<0.1 per m^2), the herbivory pressure observed can be mainly attributed to the effect of *Sarpa salpa*, a fish that is mostly distributed at shallow depths and has a certain preference for *C. cylindracea* (Tomas et al. 2011b). Nevertheless, considering that sea urchins have been previously reported feeding on *C. cylindracea* (Ruitton et al. 2006, Bulleri et al. 2009, Cebrian et al. 2011, Tomas et al. 2011a), additional negative effects on *C. cylindracea* abundance may occur in areas with high sea urchin abundances. These results where the interaction between biotic mechanisms yielded a stronger biotic resistance against the invader than the single mechanisms alone, agree with previous studies where habitat characteristics interacted with herbivory pressure to influence the overall biotic resistance of certain terrestrial ecosystems (Suwa and Louda 2012, Li et al. 2014, Zhang et al. 2018).

Also, and importantly, by following the long-term dynamics of *C. cylindracea* at the study area, we observe that the overall strength of the assemblage's biotic resistance has increased over time as the abundance of the invasive alga in assemblages subjected to higher biotic resistance (i.e., communities at depths of between 0 and 25 m), has decreased over a 10-yr period (Fig. 4). This regression, restricted to areas with high herbivore pressure, may be the result of either an increase in the abundance of the herbivores or to some herbivores becoming more efficient in consuming the invasive species as the invasion progresses (Strayer et al. 2006, Carlsson et al. 2009). However, given that the abundance of *S. salpa* has remained more or less stable during the assessed period (Coll 2020), we suspect that this herbivore has become more efficient at targeting the invader and has increased its per capita consumption rates over time (J. Santamaría, *unpublished manuscript*). Nevertheless, other

mechanisms that can increase biotic resistance, such as, for instance, allelochemical defenses deployed by native species and assemblages against the invader (Strayer et al. 2006), cannot be ruled out.

Unfortunately, in general, knowledge on whether the effects of biotic mechanisms are maintained throughout an invasion process or whether they change in relation to time since invasion is still quite limited, because most studies just assess a particular time frame in the invasion (Maron and Vilà 2001, Levine et al. 2004, Mitchell et al. 2006, Alofs and Jackson 2014, Papacostas et al. 2017). To our knowledge, our study is the first that reports the importance of exposure time for the development of resistance against invaders in natural ecosystems (but see Diez et al. 2010, Dostál et al. 2013 for assessments in common garden experiments) and highlights that increases in biotic resistance observed over time (because of increased consumer pressure, competitive impacts, or a combination of the two), could explain why invaders that have previously been considered as hypersuccessful (e.g., *Myriophyllum spicatum*, *Elodea canadensis*, *Dreissena polymorpha*, *Carcinus maenas*, *Caulerpa* species) can suffer marked reductions in population size some years after the onset of the invasion (Simberloff and Gibbons 2004, De Rivera et al. 2005, Ivesa et al. 2006, Carlsson and Strayer 2009, Bernardeau-Esteller et al. 2020). Therefore, by focusing on only a small time frame or just the beginning of an invasion, we may be underestimating the true capacity of native assemblages to develop resistance to invaders (Strayer et al. 2006, 2017, Rius et al. 2014, Papacostas et al. 2017). For this reason, the use of long-term data, despite being scarce, should be prioritized whenever possible, to assess the true effect that biotic resistance mechanisms might have on the overall invasion process.

Generally, our findings highlight the importance of considering several factors and their interaction when assessing the strength of biotic resistance mechanisms against a particular invader, especially considering that herbivory and competition are universal processes that operate across ecosystems and that naturally interact with each other (Gurevitch et al. 2000, Meiners and Handel 2000, Hambäck and Beckerman 2003). In fact, it has been proposed that herbivory reinforces competition and in turn releases the chance for coexistence, favoring those species that are better competitors (Gurevitch et al. 2000). However, despite that, in invasion ecology, the interaction between these mechanisms has been rarely assessed (but see Suwa and Louda 2012, Li et al. 2014, Zhang et al. 2018) and most studies rely on the assessment of single biotic mechanisms (Maron and Vilà 2001, Levine et al. 2004, Vilà and Weiner 2004, Kimbro et al. 2013, Papacostas et al. 2017 and references therein). This can definitely underestimate the true role of biotic processes (e.g., competition and herbivory) against invasive species and may explain why our findings, reporting a strong effect of biotic

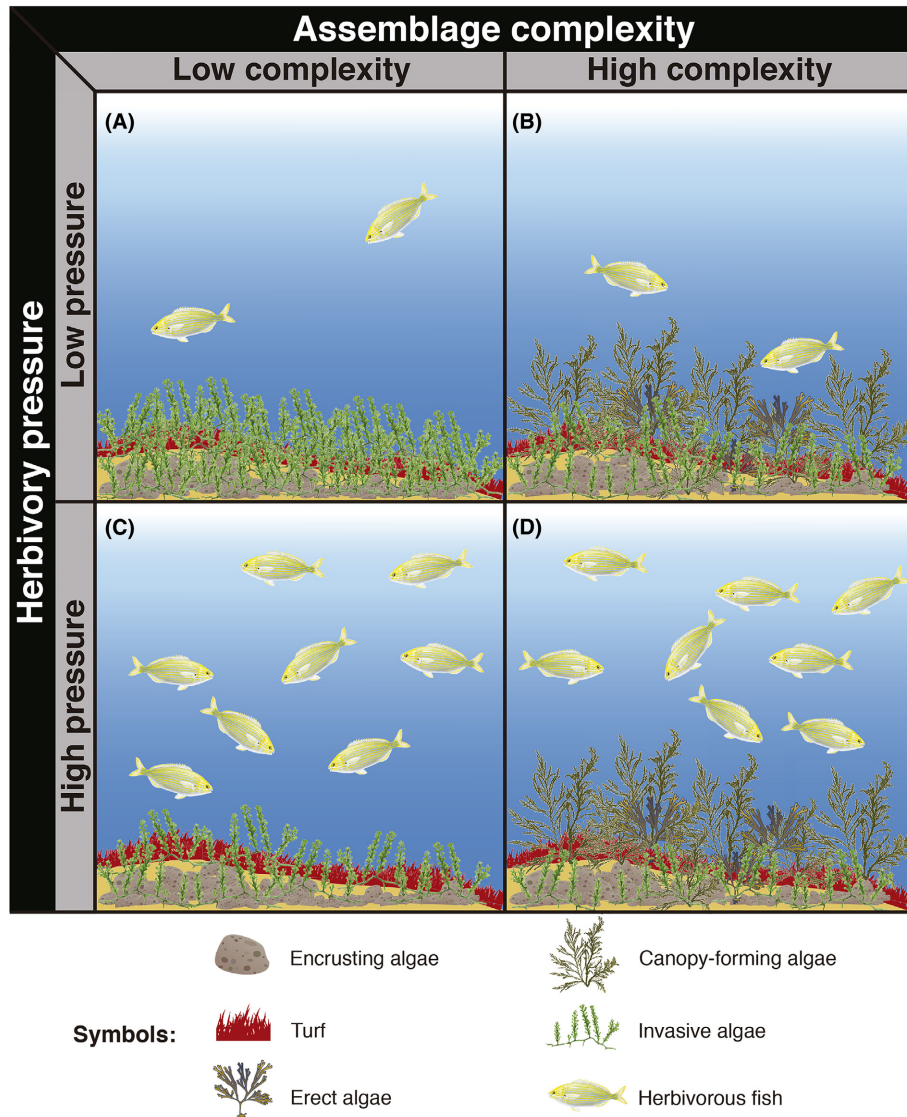


FIG. 5. Depiction of how different combinations of assemblage complexity and herbivory pressure determine biotic resistance against a marine invasive alga (e.g., *Caulerpa cylindracea*), representing four scenarios: (A) low assemblage complexity and low herbivory pressure; (B) high assemblage complexity and low herbivory pressure; (C) low assemblage complexity and high herbivory pressure and (D) high assemblage complexity and high herbivory pressure. (Algae illustrations were obtained and modified from the Integration & Application Network [IAN] Image Library [Tracey Saxby and Joanna Woerner], the IUCN, and freepik; <https://www.freepik.com/macrovectors>; the fish illustration is by João T. Tavares www.deviantart.com).

mechanisms against a successful invader, contrast with many previous studies. Actually, our results, together with those recently reported for mangrove ecosystems (Li et al. 2014, Zhang et al. 2018), where a successful invader (*Spartina alterniflora*) was limited and excluded due to the interaction between competition and herbivory, suggest that synergisms between biotic resistance mechanisms may be an important but overlooked process driving the invasion success of plant invaders (Fig. 6). In this sense, in situ field experiments, in which competition (e.g., assemblage complexity), herbivory, and

the interaction between the two can be simultaneously assessed, can provide a better understanding of the true extent of biotic resistance against an invader (Levine et al. 2004, Mitchell et al. 2006, Kimbro et al. 2013, Li et al. 2014, Enge et al. 2017, Zhang et al. 2018, Petruzzella et al. 2020) and will definitively help in the understanding of the invasion success and the dynamics of different invaders.

Finally, the findings reported in this study highlight the importance of improving our knowledge regarding the factors that influence invasive species success in

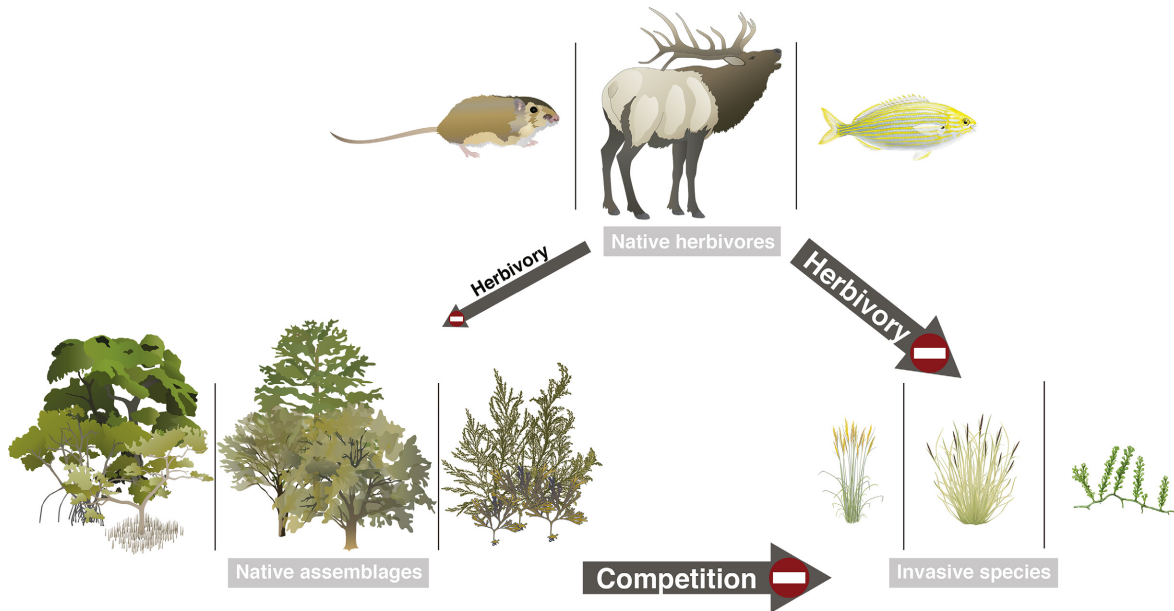


FIG. 6. Schematic representation of how the interaction between competition and herbivory might determine the overall biotic resistance against an invader. Depicted illustrations correspond to *Ratus losea*, *Cervus elaphus*, and *Sarpa salpa* as native herbivores; a mangrove forest, a temperate forest, and a marine algal forest as native assemblages; and two grasses and an alga species (here *Caulerpa cylindracea*) as invasive species. (Illustrations obtained from the Integration & Application Network [IAN] Image Library [Tracey Saxby, Kim Kraeer, and Lucy Van Essen-Fishman] and the IUCN, and the fish illustration is by João T. Tavares www.deviantart.com).

order to understand the invasion process of different species fully and adopt successful mitigation and management measures. As a practical example, although the removal of invasive algae has proven to be, in most cases, ineffective or infeasible (Epstein and Smale 2017, Giakoumi et al. 2019), results obtained in this and other studies (Bernardeau-Esteller et al. 2020) suggest that by promoting the conservation of marine habitats and herbivorous populations, we can foster biotic resistance within an ecosystem-based approach to marine environment management and contribute to the long-term control of marine invasions.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at <http://onlinelibrary.wiley.com/doi/10.1002/ecy.3440/supinfo>

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R code and data (Santamaría 2021) for this publication are available on Zenodo: <https://doi.org/10.5281/zenodo.4664432>.