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Research article

An invasive seagrass drives its own success in two invaded seas by both negatively affecting native seagrasses and benefiting from those costs

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The nature and strength of interactions between native and invasive species can determine invasion success. Species interactions can drive, prevent or facilitate invasion, making understanding the nature and outcome of these interactions critical. We conducted mesocosm experiments to test the outcome of interactions between *Halophila stipulacea*, a seagrass that invaded the Mediterranean and Caribbean Seas, and native seagrasses (*Cymodocea nodosa* and *Syringodium filiforme*, respectively) to elucidate mechanisms explaining the successful invasions. Mesocosms contained intact cores with species grown either mixed or alone. Overall, in both locations, there was a pattern of the invasive growing faster with the native than when alone, while also negatively affecting the native, with similar patterns for shoot density, aboveground and belowground biomass. In the Caribbean, *H. stipulacea* increased by 5.6 ± 1.0 SE shoots in 6 weeks when grown with the native while, when alone, there was a net loss of -0.8 ± 1.6 SE shoots. The opposite pattern occurred for *S. filiforme*, although these differences were not significant. While the pattern in the Mediterranean was the same as the Caribbean, with the invasive grown with the native increasing shoots more than when it grew alone, these differences for shoots were not significant. However, when measured as aboveground biomass, *H. stipulacea* had negative effects on the native *C. nodosa*. Our results suggest that a seagrass that invaded two seas may drive its own success by both negatively affecting native seagrasses and benefiting from that negative interaction. This is a novel example of a native seagrass species facilitating the success of an invasive at its own cost, providing one possible mechanism for the widespread success of this invasive species.

Keywords: facilitation, invasion ecology, negative interactions, plant invasion, seagrasses, species interactions

Introduction

Understanding whether species interactions play a key role in determining the success or failure of invasive species is critical for predicting future invasion success and subsequent spread (Theoharides and Dukes 2007, Schultheis and MacGuigan 2018). Species interactions may promote successful invasion as well as have negative effects on native species when the invader is competitively superior (Dueñas et al. 2018) or when the invasive species benefits from facilitation in the invaded community (McIntire and Fajardo 2014). Alternatively, invasion success may be propelled by natural or anthropogenic disturbance, with species interactions playing a minor role (King and Tschinkel 2008). Thus, to predict whether an invasive species can succeed in an invaded habitat, it is critical to understand whether the invader is a driver of its own success, a passenger of community change (defined below, sensu MacDougall and Turkington 2005), or the result of other species interactions, such as facilitation (Bertness and Shumway 1993, Bruno et al. 2003).

In one conceptual framework for successful invasion, invasive species can be 'drivers' of their own success by readily displacing native species through direct interspecific interactions, such as competition and allelopathy, or by enacting changes in surrounding environmental conditions (HilleRisLambers et al. 2010, Lambers and Oliveira 2019). The success of many invasive species has been linked empirically to superior competitive abilities (Strain and Johnson 2009, Vilà et al. 2011) that result in direct negative impacts on native species (van de Voorde et al. 2012, Zhang and van Kleunen 2019). Invasive species can be competitive dominants through superior capacities to acquire resources, such as nutrients, light or space (Goldberg et al. 1999, Gioria and Osborne 2014). Other mechanisms where invasive species drive their own success include the production of allelopathic chemicals that inhibit the growth of co-occurring native species (Callaway and Ridenour 2004, Bauer et al. 2012, Lambers and Oliveira 2019) or release of substances that change soil or water chemistry (Raniello et al. 2007, He et al. 2009, Weidenhamer and Callaway 2010, Tuya et al. 2013). Alternatively, invasive primary producers can generate positive environmental feedbacks, like enhanced nutrient cycling, that amplify their growth compared to natives (Chen et al. 2009). Thus, it is critical to explore species interactions to determine whether an invader can be a driver of its own success under certain environmental contexts.

Another conceptual framework for species invasions posits that invasive species can be 'passengers', with invasion success enhanced by disturbance or environmental change that removes native species and/or prevents their recovery (Didham et al. 2005, MacDougall and Turkington 2005). Theory predicts that disturbance-mediated passengers are usually not competitive dominants but readily colonize open space (Bulleri et al. 2010). Empirical studies have shown that early successional habitats are especially vulnerable to species invasions (Johnson et al. 2006), while the ability to invade is reduced in late-successional stands (Cunard and Lee 2009).

For example, highly disturbed habitats have been linked to the invasion and spread of grass species such as *Microstegium vimineum* (Barden 1987, Rauschert et al. 2010) as growth of the invasive plants was greater in more disturbed areas (Averill et al. 2011). Some invasive species are better at acquiring critical resources (e.g. light, nutrients) after a habitat is disturbed (D'Antonio et al. 1998). Thus, exploration of species interactions may also provide insight into whether an invasive species can be a passenger of community change under a given environmental context.

Facilitation is a positive species interaction that may influence invasion success, though these interactions are often overlooked (Bruno et al. 2003). Facilitation of species invasions can be interspecific, with native species facilitating invasive species (McIntire and Fajardo 2014), or intraspecific with invasive species exhibiting positive feedbacks (Fajardo and Siefert 2019). Facilitation by native species can enhance invasion success with a resulting decline in native species and alteration of ecosystem function (Bronstein 2009). Examples of facilitation by natives include reducing thermal stress by shading (Altieri et al. 2010), increasing access to nutrients through symbiosis (Callaway and Walker 1997) or removing/deterring predators (Stachowicz 2001). Further, native nitrogen-fixing plants can facilitate invasions by increasing available soil nitrogen, which is often a limiting resource (Kuebbing and Nuñez 2015, Kuebbing and Nuñez 2016). Alternatively, invasive plants can facilitate their own invasion by reducing native fitness (Reinhart et al. 2006, Jordan et al. 2008) or positively supporting their own growth (Vitousek et al. 1987, Ehrenfeld et al. 2001, Ehrenfeld 2003, Poulette and Arthur 2012). For example, some invasive plant species fix nitrogen or create conditions that promote fire, both of which can facilitate their own success (Stachowicz and Byrnes 2006). Therefore, it is important to examine whether facilitation plays a critical role in enhancing the success of invasive species.

Native to the Indian Ocean and the Red Sea, the seagrass *Halophila stipulacea* invaded both the Mediterranean Sea where it coexists with native seagrasses (Sghaier et al. 2014, Winters et al. 2020) and the Caribbean Sea where it is displacing native seagrasses (Steiner and Willette 2015, Smulders et al. 2017)(Fig. 1a, 2a). Thus, this seagrass is the only known marine plant to successfully invade both eastern and western hemispheres, including both temperate and tropical zones (Winters et al. 2020). Some evidence suggests that *H. stipulacea* is a passenger that can readily invade unvegetated habitats and proliferate after a myriad of disturbances that remove native species (Steiner and Willette 2015, Christianen et al. 2019, Hernández-Delgado et al. 2020, Willette et al. 2020). In contrast, *H. stipulacea* transplants successfully grew in a native-dominated seagrass bed, providing initial evidence it could be a driver (Willette and Ambrose 2012). Finally, one study suggested *H. stipulacea* can be both a passenger and a driver depending on the environmental or community context (Muthukrishnan et al. 2020). Taken together, these studies motivate more research on whether *H. stipulacea* is a passenger or driver in both invaded seas.
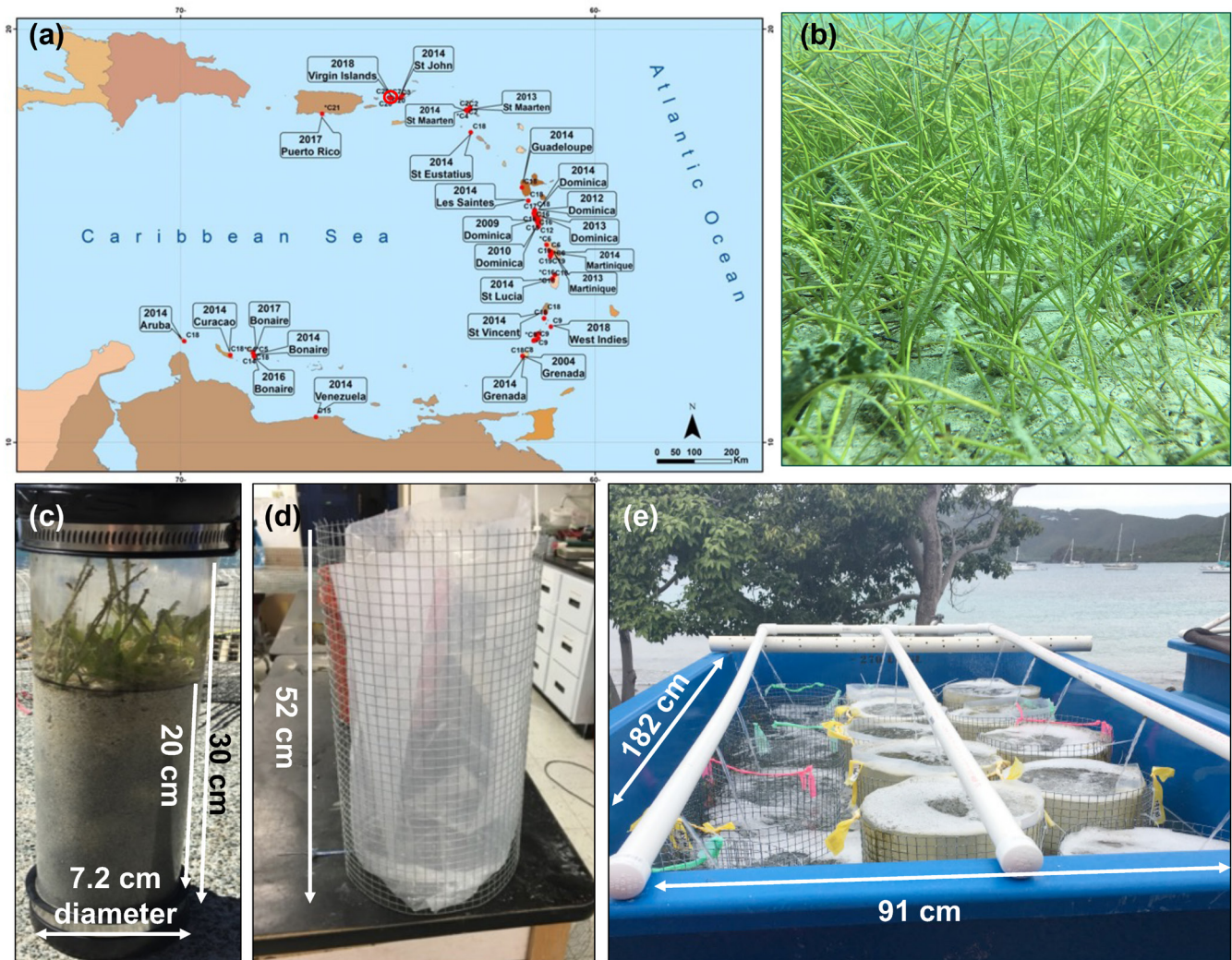
Figure 1. Experimental setup in the Caribbean. (a) Geographic distribution of published studies on *Halophila stipulacea* (red colored dots) in the Caribbean Sea labeled by year of the first report in each location (adapted from Winters et al. 2020). The white outlined circle encloses our site in Brewers Bay, St Thomas, U.S. Virgin Islands). (b) Native Caribbean seagrass *Syringodium filiforme* growing in a single species meadow and (c) experimental core of *Syringodium filiforme* mixed with the invasive *H. stipulacea*. (d) Cores from these mixed meadows were inserted into 24-l mesocosms for our experiment. (e) Mesocosms were set into large tanks and independently plumbed with flow-through seawater from Brewer's Bay for the six weeks duration of the experiment.

In this study, we examine the ecological interactions of *H. stipulacea* and native seagrasses in its two invasive ranges. Specifically, we test whether *H. stipulacea* is a driver of its own success through superior competitive abilities or whether other interspecific interactions, such as facilitation, may contribute to invasion success. Our overall approach in both seas was to quantify interactions among native and invasive seagrass species by growing them mixed and alone in flow-through mesocosms and measuring growth responses.

Material and methods

Study species and sites of donor seagrass beds

The seagrass *H. stipulacea* is native to the western Indian Ocean, eastern Africa, the Arabian Sea, the Persian Gulf and the Red Sea. *Halophila stipulacea* functions as a pioneer species in its native range (Mariani and Alcoverro 1999), where it is a short-lived opportunist that colonizes and grows rapidly in disturbed areas (Coppejans et al. 1992). *H. stipulacea* has many disturbance-mediated traits including high turnover (Azcárate-García et al. 2020), reduced longevity (Rindi et al. 1999), high tolerance to a wide range of irradiance (Lee et al. 2007), short leaf lifespan (Hemminga et al. 1999), high leaf production and rapid rhizome elongation rate (Azcárate-García et al. 2020, Winters et al. 2020).

Following the opening of the Suez Canal in 1869, *H. stipulacea* invaded the Mediterranean Sea (Den Hartog 1972) where it was first recorded in Akrotiri Bay, Limassol, Cyprus in 1968 (Lipkin 1975). Since then, *H. stipulacea* has flourished, expanding its range throughout the Mediterranean where it forms multi-species meadows with native seagrasses and algae
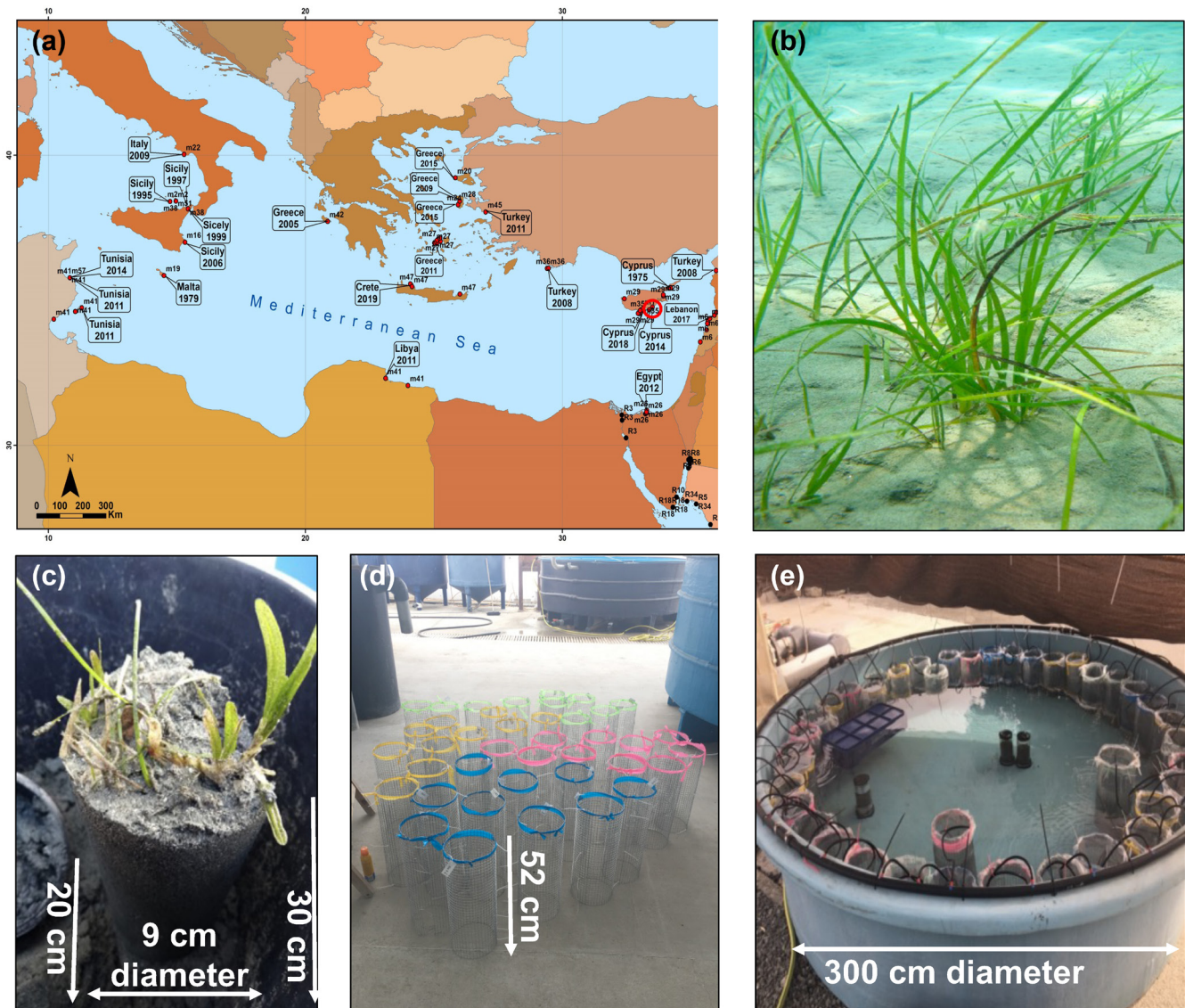
Figure 2. Experimental setup in the Mediterranean. (a) Geographic distribution of published studies on *Halophila stipulacea* (red colored dots) in the Mediterranean Sea labeled by year of the first report in each location (adapted from Winters et al. 2020). The red circle encloses the country of the site in Akrotiri Bay at Dream Café, Limassol, Cyprus. (b) Native Mediterranean seagrass *Cymodocea nodosa* growing in a single species meadow, (c) mixed with the invasive *H. stipulacea*. (d) Cores from these mixed meadows were 20 cm into the sediment deep and inserted into 17-l mesocosms. (e) Mesocosms were set into large tanks and independently plumbed with flow-through seawater from Akrotiri Bay for the six weeks duration of the experiment.

(Sghaier et al. 2014, Winters et al. 2020) (Fig. 2a). One native seagrass that co-occurs with *H. stipulacea*, *Cymodocea nodosa*, is also considered a pioneer species. It is widely distributed throughout the Mediterranean Sea (Den Hartog 1970) and can quickly colonize bare areas (Borum and Greve 2004). Although it is not as fast growing as the invasive, it also has rapid leaf turnover (Pérez and Romero 1994), rhizome production (Duarte and Sand-Jensen 1990, Rismondo et al. 1997, Cancemi et al. 2002) and rapidly generates new shoots (Terrados et al. 1997).

A second invasion of *H. stipulacea* was recorded in 2002 on the Caribbean Island of Grenada (Ruiz and Ballantine 2004). Subsequently, it expanded its range over 700 km by rapidly spreading to the north, reaching the Virgin Islands and Puerto Rico, and west, reaching Curaçao (Vera et al. 2014, Willette et al. 2014, Ruiz et al. 2017) (Fig. 1a). In contrast to the Mediterranean, *H. stipulacea* displaces native Caribbean seagrasses, often forming monoculture meadows (Ruiz and Ballantine 2004, Willette and Ambrose 2012, Willette et al. 2014). One displaced native seagrass, *Syringodium filiforme*, which is prominent along some Caribbean islands including the Virgin Islands (Kendall et al. 2004, Willette and Ambrose 2012), is also a relatively short-lived, pioneer species with high leaf turnover (Gallegos et al. 1994) and rapid shoot production (Kenworthy and Schwarzschild 1998) (Fig. 1b).

In both seas, we chose study sites comprised of natural, mixed-species, relatively low-density seagrass beds. In the Caribbean, the donor bed was in Brewers Bay, St Thomas, U.S. Virgin Islands, eastern Caribbean Sea (18°20′37″N, 64°58′46″W) between 2 and 4 m in depth. The shoot density of the native *S. filiforme* in the donor bed averaged 884.6 ± 7.6 m², which is within the overall range of density in the Virgin Islands (94–1678 shoots m⁻²) and comparable to the average shoot density in Brewers Bay (Olinger et al. 2017). It is higher than the density in Culebra, Puerto Rico, which ranged between ~77 and 300 shoots m⁻² (Hernández-Delgado et al. 2020) The shoot density of *H. stipulacea* in the donor bed averaged 786.3 ± 15.3 SE shoots m⁻², which is in the low end of the range of averages recorded across Brewers Bay (742–3850 shoots m⁻²; Olinger et al. 2017). However, it is denser than in newly-invaded Culebra, Puerto Rico, where shoot density ranged from ~20 to 400 shoots m⁻² (Hernández-Delgado et al. 2020).

In the Mediterranean, the donor seagrass bed was in Akrotiri Bay at Dream Café site, Limassol, Cyprus (34°42′20″N, 33°07′24″E) between 3 and 5 m in depth (Nguyen et al. 2018). The average shoot density of the native *C. nodosa* was 102.9 ± 12.5 SE shoots m⁻², which is below the range of shoot densities in Tunisia (394–1056 shoots m⁻²; Ben Brahim et al. 2020) and Italy (925–1925 shoots m⁻²; Cancemi et al. 2002), but comparable to what is found in Crete (115–159 shoots m⁻²; Apostolaki et al. 2019). The invasive seagrass was 231.9 ± 3.9 SE shoots m⁻², which was

lower than natural densities in other Mediterranean sites, including Akrotiri Bay, where averages ranged from ~ 800 to 2000 shoots m⁻² (Nguyen et al. 2020a), and Italy with an average of 10 500 shoots m⁻² (Gambi et al. 2009). However, density of the donor bed appeared typical of other sites around Limassol (Chiquillo et al. unpubl.).

Experimental design

St Thomas, USVI, Caribbean experiment
We explored the nature of species interactions in the more recent range expansion into the Caribbean with three experimental treatments: 1) native and invasive species mixed in ambient densities, 2) invasive species alone and 3) native species alone (Fig. 3a). This mesocosm experiment was conducted for 6 weeks from 1 February to 21 March 2017 at the University of Virgin Islands (USVI), St Thomas, Caribbean.

To contextualize our experimental conditions, we measured water temperature and light in the donor seagrass beds and mesocosms. Light and temperature in the field were measured with a pendant HOBO Temperature/Light 64K logger (Model UA-002-64) placed on the benthos within the donor seagrass canopy between 2 and 4 m depth. In the field, readings were taken every 15 min for 24 hours the day before and 3 days after the experiment. During the experiment, a logger was placed on the sediment of 1 experimental unit (see below for full description) and both temperature and light measurements were recorded every 15 min for 41 days (8 Feb–21

(a) Newly invaded range- St. Thomas, U.S. Virgin Islands, Caribbean

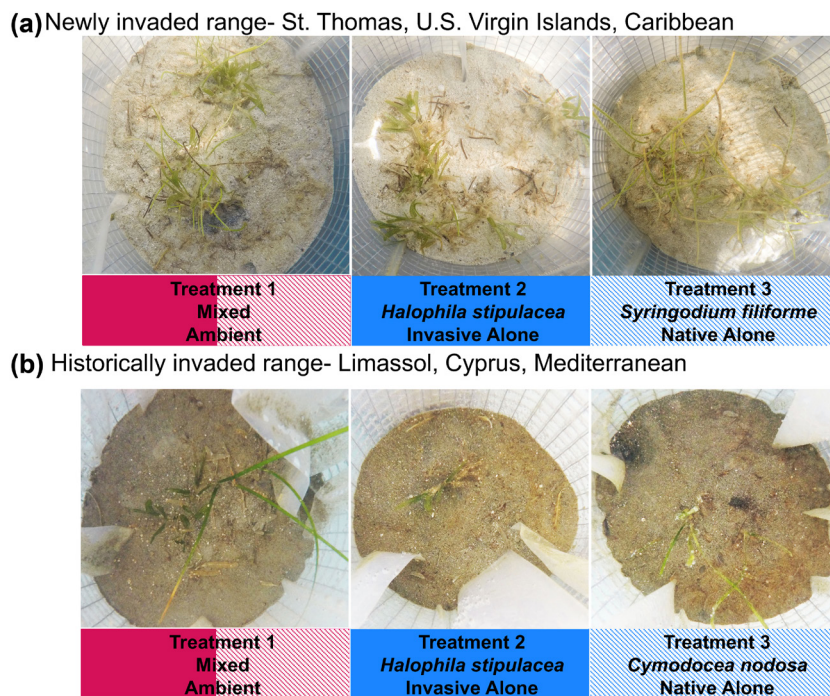**(b)** Historically invaded range- Limassol, Cyprus, Mediterranean

Figure 3. (a) Top view of experimental design in the newly-invaded Caribbean. Treatment 1 ambient represents mixed conditions where both species are present. Treatment 2, Hs, is the invasive *H. stipulacea* alone. Treatment 3, Sf, is native *S. filiforme* alone. Each of the three treatments has five replicates (n = 15). (b) Top view of experimental design in the historically-invaded Mediterranean. Treatment 1, ambient, represents mixed conditions where both species are present. Treatment 2, Hs, is the invasive *H. stipulacea* alone. Treatment 3, Cn, is the native *C. nodosa* alone.

March); mesocosms and loggers were cleaned of epiphytes at least every other day. The logger remained in the same experimental unit with weekly re-randomization in the location of this unit within the water table. Following (Apostolaki et al. 2014), daytime light levels were calculated by averaging light intensity measures from 1 hour after sunrise until 1 hour before sunset in both the field and mesocosms.

To create the three treatments, we constructed outdoor mesocosms consisting of intact cores (sediment with seagrass and epiphytes) from a natural, mixed community of *H. stipulacea* and *S. filiforme*. Individual experimental mesocosms were 24-l cylinders that were 52 cm in height and 24 cm in diameter and lined with clear 46 × 61 cm (width × height) rectangular polyethylene bags (Fig. 1d–e). Intact cores were collected by SCUBA using clear polycarbonate cores 30 cm in height and 7.2 cm in diameter (Fig. 1c). Cores were collected haphazardly approximately 1 m apart. Two intact cores were placed into each experimental mesocosm. To ensure roots were covered and there was sediment into which they could expand, we filled the spaces between and around the cores in each experimental mesocosm with sand from the collection bed until the sediment depth was the same as the cores (~15 cm in depth).

We counted initial shoots in each experimental unit. Although we did not count rhizome apical meristems initially, we counted number of apical shoots at the end of the experiment, as including sufficient numbers can be a concern for *S. filiforme* (Schwarzschild and Zieman 2008). However, another study showed that 10 cm diameter cores contained enough rhizome apical meristems of *H. stipulacea* to grow as transplants (Willette and Ambrose 2012).

We did not measure initial aboveground and belowground biomass within the experimental units of the treatments as these methods are disturbing or destructive. Instead, we randomly collected an additional five cores to estimate initial values for these parameters. We cleaned sediment and debris from the seagrasses, then separated them into aboveground (blades and shoots) and belowground (roots and rhizomes) portions, spun each portion for 1 min in a salad spinner to remove excess water and wet weighed (g) them.

To establish our treatments, we left five experimental mesocosms as mixed communities, removed *S. filiforme* from 5 experimental mesocosms, and removed *H. stipulacea* from the remaining five experimental mesocosms. To control for disturbance effects, we disturbed sediment by hand digging to the rhizome level in mixed treatments to mimic the disturbance caused by the removal of seagrass. We randomly assigned mesocosms to different positions in a large flow-through tank (2 × 0.6 × 1 m) that acted as a water bath to keep a constant temperature among replicates. We filled each mesocosm to the top with unfiltered seawater and used an overflow system to replace water. Each mesocosm had an independent water source with a constant seawater flow; there were approximately 16 turnovers/per day. Positions of the mesocosms within the large tank were re-randomized every seven days to account for any spatial variation within the large tank. To minimize the impacts of other species interactions (e.g. herbivory) we observed mesocosms every other day and removed invertebrates as needed. After six weeks, we removed mesocosms from the tank, counted the live shoots in each mesocosm, and calculated the net change in shoot density for each species present. We then measured the final aboveground and belowground wet biomass as described above.

Limassol, Cyprus, Mediterranean experiment

To examine the effects of species interactions on the growth of native and invasive seagrasses in the historically-invaded range, we conducted a similar mesocosm experiment as described above for the Caribbean in Cyprus, in the Mediterranean (Fig. 3b). There are six key differences between the Caribbean and this Mediterranean experiment. First, we chose the native seagrass, *C. nodosa* (Fig. 2b), a common seagrass in the Mediterranean (Pérez and Romero 1994). Second, due to differences in facilities, the mesocosm, tank and core sizes were different. Mesocosms (n = 40) were smaller 17-l cylinders (compared to the 24-l used in the Caribbean) that were 52 cm in height and 20 cm in diameter lined with clear 31 × 20 × 61 cm (width × depth × height) three-dimensional (rather than rectangular as in the Caribbean) polyethylene bags (Fig. 2d–f). We randomly assigned mesocosms to a flow-through circular tank (0.8 m depth × 3 m diameter). Cores tubes were slightly larger, at 30 cm in height and 9 cm in diameter, made from clear polycarbonate (Fig. 2c). Thus, we placed only one intact core (sediment with seagrass and epiphytes) in each mesocosm (in the Caribbean, we placed two cores in each mesocosm). Third, we increased the number of experimental replicates to 10. Fourth, initial shoot densities and biomasses were lower than in the Caribbean due to differences in ambient densities (Results). Fifth, temperature and light measurements in the mesocosms were recorded every 3 min for 10 days during the experiment using HOBO loggers, and data were processed as above. However, we were unable to record field measurements of these parameters. And sixth, we added another treatment to test for density effects, where native and invasive species were grown mixed, but we reduced densities by one-half (methods and results of this treatment are presented in the Supporting information).

This mesocosm experiment was maintained using methods described for the Caribbean and ran for six weeks from 15 October to 3 December 2018 in Larnaka, Cyprus, at the Cyprus Marine Aquaculture Center of the Department of Fisheries and Marine Research, Ministry of Agriculture, Rural Development and Environment in Meneou, Cyprus.

Response variables and statistical analyses

Change in shoot density was calculated as the difference in the number of shoots over the six-week experiment; shoots were counted both initially (I) and finally (F) and change was calculated as F–I. Because initial above and belowground biomass were taken from cores not used in the experiment, we did not calculate change per mesocosm. Thus, the response variables used for these metrics were final measures, with initial values indicated on each graph for visual comparison.

We used R stats packages to test if data residuals were normal using the Shapiro–Wilk test *shapiro.test* and for homoscedasticity of variances using Bartlett's test *bartlett.test*' and the *leveneTest* in 'car' package (Fox and Weisberg 2019). In the Caribbean, final belowground biomass met assumptions untransformed, while changes in shoot density were squared and final aboveground biomasses were square root transformed. In the Mediterranean, changes in shoots and final aboveground biomass data were square root transformed and belowground biomass data were log transformed to increase conformance to normality.

Change in shoots, final aboveground biomass and final belowground biomass were analyzed using two-factor ANOVAs with two categorical predictor variables. Predictor variables were species (native versus invasive), species treatment (alone versus mix) and their interaction, using the 'aov' stats package in R ver. 3.6.1 (<www.r-project.org>). Following a significant interaction in ANOVA, we used Tukey's multiple comparison test to determine which treatments were significantly different from each other using the package and function 'lsmeans' in R (Lenth 2016). Tukey's post hoc adjusted p-values less than 0.05 were considered to be significantly different between treatments.

Results

St Thomas, USVI, Caribbean experiment

Water temperature in the field ranged between 22 and 26°C while temperatures recorded in our mesocosm ranged slightly higher, between 24 and 28°C. Average light in the field from January to March was 428 ± 67 Lux (8.13 ± 1.27 µmol m^{-2} s^{-1}; see Thimijan and Heins (1983) for conversion formula) with a maximum intensity of 6200 Lux (117.8 µmol m^{-2} s^{-1}). The average light intensity in the mesocosms was 1177 ± 37 Lux (22.3 ± 0.7 µmol m^{-2} s^{-1}) with a maximum light intensity of 8266 Lux (157.1 µmol m^{-2} s^{-1}). Although light intensity was overall higher in our mesocosm than in the field, average light intensities of shallow tropical seagrass beds reported in another Caribbean study were also much higher, ranging from 1566 to 7577 Lux (29.75–143.96 µmol m^{-2} s^{-1}) (Kantún-Manzano et al. 2018).

Initial seagrass shoot densities averaged 25.6 ± 0.5 SE shoots per mesocosm for the invasive *H. stipulacea* and 28.8 ± 0.2 SE shoots for the native *S. filiforme*, with no difference between mixed versus alone treatments for either species (*S. filiforme* mixed versus *S. filiforme* alone – Welch's t-test, t = 0, df = 8, p = 1; *H. stipulacea* mixed versus *H. stipulacea* alone Welch's t-test, t = 0, df = 6.2161, p = 1). Initial biomass is indicated with dashed horizontal lines in Fig. 4b–c. The initial aboveground biomass for the invasive averaged 1.2 ± 0.3 SE g while the native was 2.3 ± 0.6 SE g. For the invasive, initial belowground biomass was 2.1 ± 0.5 SE g and for the native it was 6.1 ± 1.8 SE g. Although we did not count rhizome apical meristems initially, final counts were 8.0 ± 1 SE for *H. stipulacea* and 6.0 ± 0.7 SE shoots of *S. filiforme*.
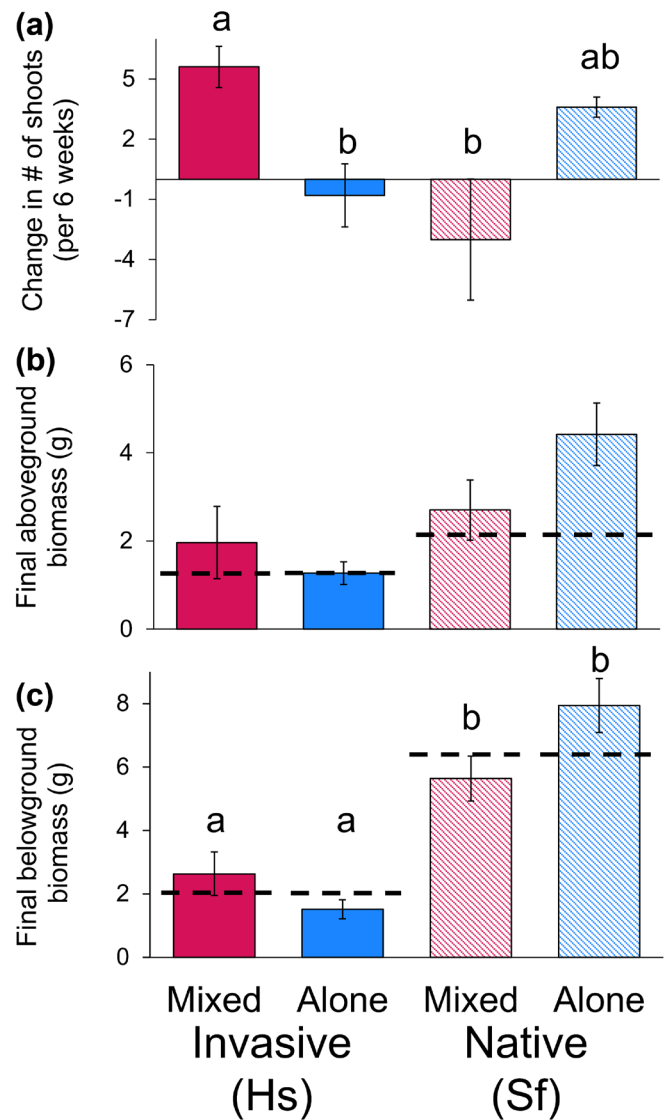

Figure 4. Results of the mesocosm experiment in the Caribbean for (a) the change in number of shoots after six weeks, (b) final aboveground biomass and (c) final belowground biomass. Pink represents the ambient, mixed treatment for both species. Blue is Hs, the invasive *H. stipulacea* alone and stippled blue is Sf for *S. filiforme* alone. Bars represent mean ± standard error. Dashed lines represent estimates of initial biomass. Tukey's post hoc test reveal pairwise comparisons across treatments and different letters indicate significant differences (p < 0.05), while bars with the same letters are not significantly different from each other.

The invasive seagrass increased shoot density over the 6-week experiment when grown in a mixed community but lost shoots when grown alone (Fig. 4a). This pattern did not occur for the native seagrass, *S. filiforme*, resulting in a significant statistical interaction (Table 1a). The average increase in number of *H. stipulacea* shoots was 5.6 ± 1.0 SE shoots when grown mixed with the native, an increase of 22.0% ± 4.3 SE from initial. However, when grown alone, there was an overall loss in the number of shoots, with an average decline of −0.8 ± 1.6 SE shoots, a −3.9% ± 5.8 SE decrease. Tukey's post

Table 1. (a–c) Statistical results of 2 factor ANOVAs for the Caribbean experiment. p-values in bold are significant.

Source of variation	df	SS	MS	F-value	p-value
(a) Response variable: Difference in shoots after six weeks					
Treatment	1	3302	3302	0.408	0.532
Species	1	11472	11472	1.417	0.251
Treatment × Species	1	137614	137614	17.004	**0.001**
Residuals	16	129492	8093		
(b) Response variable: Final aboveground biomass					
Treatment	1	0.114	0.1145	0.529	0.478
Species	1	2.059	2.0590	9.514	**0.007**
Treatment × Species	1	0.537	0.5369	2.481	0.135
Residuals	16	3.463	0.2164		
(c) Response variable: Final belowground biomass					
Treatment	1	1.76	1.70	0.897	0.358
Species	1	111.16	111.1	56.716	**< 0.0001**
Treatment × Species	1	14.64	14.6	7.469	**0.015**
Residuals	16	31.36	1.96		

hoc confirmed the positive effect of *S. filiforme* on growth of *H. stipulacea*, as the change in number of shoots for *H. stipulacea* was significantly higher when grown mixed compared to alone. In contrast, there was a trend for the native species, *S. filiforme*, to lose shoots when mixed with the invasive and increase shoots when grown alone. However, Tukey's post hoc test did not detect a significant difference between alone and mixed treatments for *S. filiforme*, likely due to high variability in the mixed community.

There was a significant difference in final aboveground biomass between the two species of seagrass (Table 1b), with aboveground biomass of the invasive species being about half that of the native (averaged across both treatments where each species occurred; Fig. 4b). In contrast, there was no effect of being grown mixed or alone or an interaction between factors. While the overall pattern for aboveground biomass was similar to that for shoots, this pattern did not produce a significant interaction (Table 1b).

The invasive seagrass also had more final belowground biomass when it grew in a mixed community than when growing alone and this pattern did not occur for the native (Fig. 4c), resulting in a significant interaction (Table 1c). However, despite the significant interaction in the ANOVA, the more conservative Tukey's post hoc, with adjusted p-values for multiple comparisons, detected no differences in the mixed versus alone treatments for either species.

Limassol, Cyprus, Mediterranean experiment

In the experimental outdoor mesocosms, the temperature ranged from 23 to 25°C and the average light intensity was 417 ± 7 SE Lux (7.92 ± 0.13 SE µmol m^{-2} s^{-1}) with a maximum intensity of 2670 Lux (50.73 µmol m^{-2} s^{-1}). While we did not measure light and temperature in the field, Nguyen et al. (2020a) measured temperature at the same site and during the same months (Oct–Dec) in Akrotiri Bay and found it ranged from 21 to 26°C. In addition, these mesocosm values were similar to our field measurements in St Thomas, Caribbean, although the maximum temperature was lower. Further, other studies found the average light intensity

of seagrass meadows in the Balearic Islands, Mediterranean ranged between 5442 and 6711 Lux (103.4–127.51 µmol m^{-2} s^{-1}) at depths of 5 to 12 m (Hendriks et al. 2014), albeit these islands are more than 2700 km from Cyprus.

Initial seagrass shoot density (mixed and alone) averaged 5.9 ± 0.1 SE shoots for the invasive *H. stipulacea* and 3.0 ± 0.2 SE shoots for the native *C. nodosa*. A t-test comparing mean initial shoot densities in mixed versus alone treatments found no differences (*C. nodosa* mixed versus *C. nodosa* alone Welch's t-test, t = 0.60609, df = 10.802, p = 0.557; *H. stipulacea* mixed versus *H. stipulacea* alone Welch's t-test, t = 1, df = 9, p = 0.3434). Initial aboveground biomass for the invasive *H. stipulacea* averaged 0.47 ± 0.1 SE g, while the native *C. nodosa* was 0.43 ± 0.1 SE g. Initial belowground biomass for the invasive was 1.2 ± 0.2 SE g and the native was 2.5 ± 0.6 SE g. Initial biomasses are indicated with dashed horizontal lines in Fig. 5b–c. Initially there were 1.3 ± 0.3 apical shoots for *Halophila*, and 1 ± 0.8 SE apical shoots for *Cymodocea*.

There was a significant statistical interaction between species and treatment for change in shoot density in the Mediterranean (Table 2a). The overall pattern was the same as in the Caribbean, with the invasive *H. stipulacea* increasing shoot density when grown mixed with the native but not changing when grown alone (Fig. 5a), and the native only increasing shoots when alone, generating the interaction). However, the only significant difference in means identified with the Tukey post hoc was the difference between species when both species were grown together. *H. stipulacea* growth was 3.7 ± 1.3 SE shoots when grown with the native, an increase of 59.2% ± 23.8 SE from initial. In contrast, the native *C. nodosa* lost shoots when mixed with the invasive, with an average loss of −1 ± 0.4 SE shoots, or −29.2% ± 13.7 SE.

A significant interaction between species and treatment (Table 2b) was generated by a similar pattern as for shoots of more aboveground biomass when the invasive seagrass grew in mixed treatments than alone while the native had more aboveground biomass when alone than mixed (Fig. 5b). Post hocs revealed a negative effect of the invasive on the native;
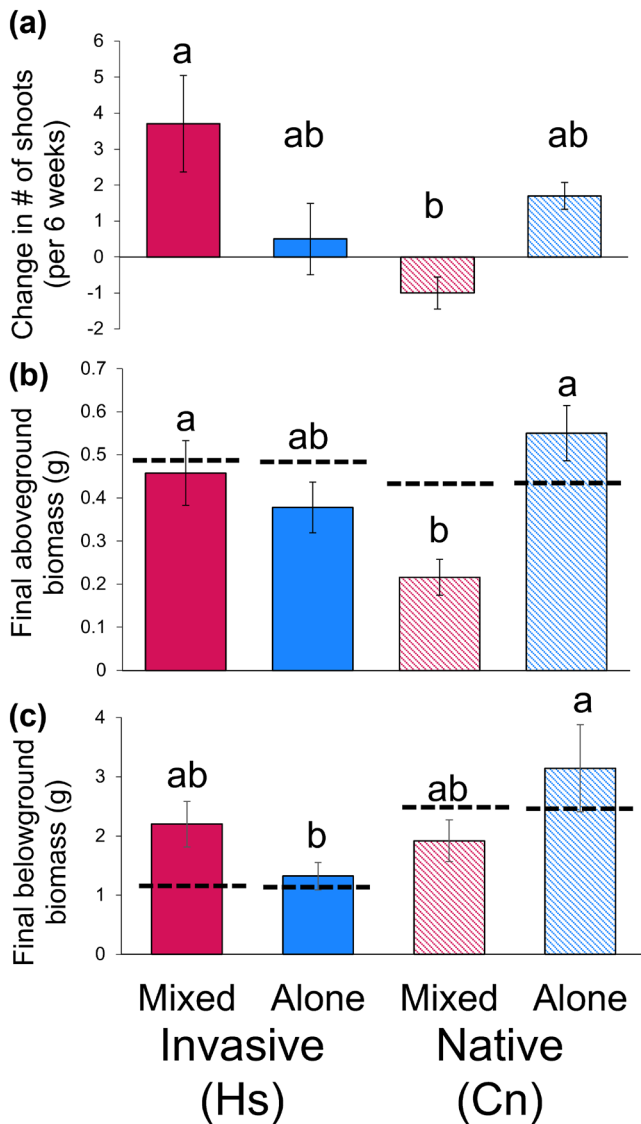
Figure 5. Results of the mesocosm experiment in the Mediterranean for (a) the percent change in number of shoots after six weeks, (b) final aboveground biomass and (c) final belowground biomass in grams. Pink represents the ambient, mixed treatment for both species. Blue is Hs for invasive *H. stipulacea* alone and stippled blue is Cn for native *C. nodosa* alone. Bars represent mean ± standard error. Dashed lines represent estimates of initial biomass and dotted lines are calculated values of the initial biomass assuming we removed exactly half.

when the native *C. nodosa* grew mixed with the invasive its average aboveground biomass was 0.18 ± 0.03 SE g, a decrease of over 50% compared to initial values (initial=0.43 ± 0.11 SE g). In comparison, when the native grew alone, its average aboveground biomass of 0.55 ± 0.06 SE g was significantly higher than the native in mixed treatments. When the two species were grown together, they also had significantly different aboveground biomass, with a final average aboveground biomass of the invasive *H. stipulacea* of 0.46 ± 0.08 SE g, which was similar to estimates for initial biomass (0.47 ± 0.06 SE g).

Final biomass of the invasive *H. stipulacea* had an overall pattern similar to the previous two metrics, with higher values when grown mixed with the native than when grown alone. The native species showed the opposite pattern (Fig. 5c), resulting in a significant interaction (Table 2c). However, for this metric, the only difference in means detected by the Tukey was between the two treatments where the species were grown alone. When the invasive *H. stipulacea* was grown alone its final belowground biomass was 1.32 ± 0.23 SE g. In contrast, when the native seagrass *C. nodosa* grew alone its belowground biomass was 3.15 ± 0.74 SE g). All results of the reduced density treatments can be found in the Supporting information.

Discussion

Controlled mesocosm experiments in the Caribbean demonstrate that *H. stipulacea* had greater growth, most strongly demonstrated as an increase in shoots, in the presence of native seagrasses and lower growth alone. A similar, albeit less strong pattern occurred in the Mediterranean. Taken together, these results suggest that native species may facilitate the success of invasive *H. stipulacea* populations. While positive effects of native on invasive species have been documented in terrestrial and other marine systems (McIntire and Fajardo 2014, Cavieres 2021), ours is the first study to document positive effects for invasive seagrasses. In contrast, native seagrasses performed better alone than in the presence of *H. stipulacea*, with the strongest evidence provided by aboveground biomass in the Mediterranean. Other studies also report variability in the strength of impacts of invasion across habitats, scales and response variables (for a review see Pyšek et al. 2012). However, overall, our study shows there is a positive effect of the native on the invasive that is coupled with a negative effect of the invasive on the native seagrass.

That this same pattern occurred with different native species and in two invaded seas at opposite ends of the world implies this coupled facilitative relationship for the invasive and antagonistic relationship for the native may be an important aspect of the invasion ecology of *H. stipulacea*. It also implies that this seagrass is a driver of its own invasion success. Willette and Ambrose (2012) demonstrated the ability of *H. stipulacea* to expand when transplanted into a native *S. filiforme* bed, but this work was only in the Caribbean and, as a field experiment, did not identify species interactions as the underlying mechanisms of success. Knowing that *H. stipulacea* can be a driver of its invasion success is critical because it implies that *H. stipulacea* does not require disturbance and recently opened space to succeed but instead can invade and thrive in intact native communities. These findings are key for managers to understand and predict the future spread of this very successful invasive species worldwide, as human impacts will only increase invasions in the Anthropocene (Ricciardi and Cohen 2007).

Table 2. (a–c) Statistical results of 2 factor ANOVAs for the Mediterranean experiment. p-values in bold are significant.

Source of variation	df	SS	MS	F-value	p-value
(a) Response variable: Difference in shoots after six weeks (Cn versus Hs Mediterranean)					
Treatment	1	0.014	0.014	0.034	0.854
Species	1	0.836	0.836	2.029	0.163
Treatment × Species	1	4.325	4.325	10.495	**0.003**
Residuals	36	14.834	0.412		
(b) Response variable: Final aboveground biomass (Cn versus Hs Mediterranean)					
Treatment	1	0.136	0.13602	5.08	**0.030**
Species	1	0.0126	0.0126	0.471	0.497
Treatment × Species	1	0.2815	0.28148	10.514	**0.003**
Residuals	36	0.9638	0.02677		
(c) Response variable: Final belowground biomass					
Treatment	1	0.0017	0.0017	0.028	0.868
Species	1	0.1850	0.1850	3.114	0.086
Treatment × Species	1	0.5320	0.5320	8.954	**0.005**
Residuals	36	2.1390	0.0594		

Native species as facilitators of invasion success at their own cost

Our results demonstrate that native seagrasses can enhance growth of *H. stipulacea* when grown in mixed communities. Similar examples of native species facilitating invasive species are well-documented in terrestrial and other marine systems (Zabin and Altieri 2007, Bronstein 2009, Flory and Clay 2010, Sueiro et al. 2013, Gribben and Byers 2020), but not for invasive seagrasses. Importantly, this facilitation occurred across both historically (the Mediterranean Sea) and much more recently (the Caribbean Sea) invaded seas, albeit its strength and importance were not consistently strong across all response variables and systems (Pyšek et al. 2012). Taken together, our results imply that the positive effects of native seagrass species can contribute to the invasion success of *H. stipulacea* (McIntire and Fajardo 2014, Cavieres 2021), providing one possible explanation of the widespread success of this invader.

Our results also demonstrate that the invasive seagrass has the potential to negatively impact the native species. There are several possible mechanisms underlying the negative impacts on native species that may be coupled with positive effects on the invasive. One is that *H. stipulacea* carries microbial associations (Gribben et al. 2017) that release compounds that negatively affect the native species (Orr et al. 2005); this negative effect may release resources for use by the invasive (Uyà et al. 2020) or change sediment chemistry toward conditions that only the invader can tolerate (Morrison et al. 2000). It is also possible that *H. stipulacea* can form positive associations with novel epibionts that enhance nutrient or vitamin acquisition at the cost of the native, as seen in the invasive seaweed *Gracilaria vermiculophylla* (Saha et al. 2016). Alternatively, positive associations that favor invasive species in terrestrial, grasslands may enhance nutrient acquisition (Tharayil et al. 2009) or decrease positive microbial associations with other plants (Mummey and Rillig 2006). The release of allelopathic chemicals (Callaway and Ridenour 2004) is another invasive strategy that results in negative effects on native species of terrestrial grasses (Greer et al. 2014). It is also possible that these negative effects release limited resources or change sediment chemistry toward conditions only tolerable for invasive species, facilitating their success. All of these potential mechanisms have yet to be explored for this invasive seagrass and deserve further attention.

Another possible mechanism underlying the positive effects of the native on the invasive seagrass is that *H. stipulacea* may be able to take advantage of recycled nutrients, deriving a nutritional benefit from the dying native seagrasses. Support for this explanation comes from Di Genio et al. (2021) who demonstrate *H. stipulacea* can grow on dead matte (a bioconstruction comprised of large quantities of buried organic debris) of the native seagrass *Posidonia oceanica*, suggesting decomposition as a potential source of limiting nutrients (Gambi et al. 2009). Moreover, one study found that a *Halophila*-dominated bed was nutrient limited but a native *Thalassia testudinum* dominated bed was not (Muthukrishnan et al. 2020), suggesting that *H. stipulacea* may more efficiently sequester nutrients.

Despite similar results in both invasive ranges, it is possible that the positive effect of the native on the invasive was an artifact of the disturbance due to the experimental removal of the native. While we aimed to disturb all mesocosms equally to reduce the likelihood of this artifact, we cannot know this was entirely efficacious. However, we argue that this explanation is unlikely as *H. stipulacea* grew vigorously in the reduced density treatment (Supporting information), which was also created by removing seagrass, suggesting that disturbance alone did not produce these effects.

H. stipulacea as a driver of its own invasion success

Our results demonstrate that *H. stipulacea* can be a driver of its own success, at least in the relatively low-density seagrass beds in both the Mediterranean and Caribbean that we studied. This finding adds to the body of work documenting that invasive species can be drivers (reviewed by Thomsen et al. 2011). Our results contrast previous observational studies of active invasion fronts in the Caribbean that indicate that *H. stipulacea* is a passenger, opportunistically establishing

and expanding into open habitats or areas of recent disturbance. For example, field surveys in Bonaire found that *H. stipulacea* expands into seagrass-dominated sites only after first settling in deeper areas devoid of seagrass (Debrot et al. 2012, Smulders et al. 2017) or where natural and anthropogenic disturbances created open space (Smulders et al. 2017). Similarly, *H. stipulacea* in Dominica and St. John expanded in locations that were devoid of native seagrasses (Willette et al. 2014). Combined, these studies support the hypothesis that *H. stipulacea* expansion can be mediated by the presence of open spaces or disturbances such as turtle grazing, storms, damage by propellers or anchors, eutrophication and bioturbators (Steiner and Willette 2015, van Tussenbroek et al. 2016, Willette et al. 2020).

However, results from our study clearly demonstrate that *H. stipulacea* can also be a driver of its own success in both invasive ranges. A previous review (Winter et al. 2020) reports *H. stipulacea* commonly exists in mixed-species seagrass communities, consistent with our finding that *H. stipulacea* can be successful in intact native communities. Once present, it can negatively impact native seagrasses, such as in Tunisia where the expansion of *H. stipulacea* correlates with a dramatic decrease in shoot density of the native seagrass, *C. nodosa*, even in the absence of disturbance (Sghaier et al. 2014), supporting the driver hypothesis. Willette and Ambrose (2012) also provide evidence that Caribbean *H. stipulacea* can expand into existing seagrass beds. This pattern of a species being a driver of their own success at the expense of the native species is similar to studies in terrestrial (Grman and Suding 2010) and other marine systems (Gribben and Byers 2020). For example, an invasive weed *Acacia dealbata* increases net production when growing in native terrestrial plant communities (Le Maitre et al. 2011). Similarly, in plant communities of freshwater lakes several invasive species increased in the presence of native plants (Capers et al. 2007). These field studies combined with the results of our mesocosm experiments provide clear and strong support that *H. stipulacea* can be a driver of its own success in both regions it invaded.

Whether *H. stipulacea* acts as a driver of its own success, is a passenger of community change, or both, may depend on the ecological or environmental context of the invaded community (Muthukrishnan et al. 2020). A plethora of experiments in other systems confirmed that being a driver can be context dependent, though most studies focused on the context of the invader rather than the invaded community (reviewed by Thomsen et al. 2011, 2014). However, context dependence of the invaded community could explain differences in impacts of the invasive marine ascidian *Ciona robusta* that decreased species diversity in one invaded region, but not another (Robinson et al. 2017) and an invasive alga that only impacts some kelp communities (Epstein et al. 2019). Previous studies found that in dense and healthy native seagrass beds *H. stipulacea* may play the role of a passenger, invading only after disturbances, such as hurricanes (Hernández-Delgado et al. 2020) or intensive grazing by a megaherbivore (Christianen et al. 2019), reduce or remove the native species. However, shoot densities in the seagrass beds used in our study had densities on the lower end of reported ranges. Thus, our results imply that in the context of relatively sparse native communities, *H. stipulacea* can be a driver, invading and replacing native species, even without disturbance.

The complex role of *H. stipulacea* as a driver that is also facilitated by native species is important because recent models of the Mediterranean Sea predict that this invasive species will keep expanding as climate change produces more tropical conditions (Beca-Carretero et al. 2019, Nguyen et al. 2020b). However, the results of our study suggest that predictions of future invasiveness may also need to consider the density of the native seagrass in the 'receiving' habitat, which also may be affected by climate change. Based on our findings, we predict that *H. stipulacea* will spread more vigorously in habitats that may be marginal for other seagrasses, without the need for a disturbance that removes native seagrass species.

Concluding statement

Our results demonstrate *H. stipulacea* may not require disturbance or community change to invade but can be a driver of its own success in low-density native seagrass communities. Worldwide, native seagrasses are declining (Orth et al. 2006) due to multiple stressors in the Anthropocene (i.e. climate change, coastal development and degraded water quality) (Waycott et al. 2009). These declines, and associated low-density states, may make native seagrass habitats more invadable, providing one explanation of the broadening invasion success of *H. stipulacea*. As such, the sustainability of native seagrass beds and the ecological goods and services they provide may hinge on maintaining dense, healthy seagrass beds capable of fending off invasions.

Author contributions

Kelcie L. Chiquillo: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Funding acquisition (equal); Investigation (lead); Methodology (lead); Project administration (lead); Software (lead); Visualization (lead); Writing – original draft (lead); Writing – review and editing (equal). **Paul H. Barber**: Conceptualization (equal); Funding acquisition (equal); Methodology (equal); Resources (equal); Supervision (equal); Writing – review and editing (equal). **Marlen I. Vasquez**: Funding acquisition (equal); Investigation (equal); Methodology (equal); Project administration (equal); Resources (equal); Validation (equal); Writing – review and editing (equal). **Edwin Cruz-Rivera**: Conceptualization (supporting); Funding acquisition (equal); Investigation (equal); Methodology (equal); Project administration (equal); Resources (equal); Validation (equal); Writing – review and editing (equal). **Demian A. Willette**: Conceptualization (supporting); Resources (equal); Validation (equal); Writing – review and editing (equal). **Gidon Winters**: Investigation (equal); Methodology (equal); Project administration (equal); Resources (equal); Validation (equal); Writing – review and editing (equal). **Peggy Fong**: Conceptualization (equal); Investigation (equal); Methodology (equal); Resources (equal); Supervision (lead); Validation (equal); Visualization (equal); Writing – original draft (equal); Writing – review and editing (lead).

Data availability statement

Supporting information

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