Mechanisms Influencing the Spread of a Native Marine Alga

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Abstract

Like invasive macrophytes, some native macrophytes are spreading rapidly with consequences for community structure. There is evidence that the native alga Caulerpa filiformis is spreading along intertidal rocky shores in New South Wales, Australia, seemingly at the expense of native Sargassum spp. We experimentally investigated the role physical disturbance plays in the spread of C. filiformis and its possible consequences for Sargassum spp. Cleared patches within beds of C. filiformis (Caulerpa habitat) or Sargassum spp. (Sargassum habitat) at multiple sites showed that C. filiformis had significantly higher recruitment (via propagules) into its own habitat. The recruitment of Sargassum spp. to Caulerpa habitat was rare, possibly due in part to sediment accretion within Caulerpa habitat. Diversity of newly recruited epibiotic assemblages within Caulerpa habitat was significantly less than in Sargassum habitat. In addition, more C. filiformis than Sargassum spp. recruited to Sargassum habitat at some sites. On common boundaries between these two macroalgae, the vegetative growth of adjacent C. filiformis into cleared patches was significantly higher than for adjacent Sargassum spp. In both experiments, results were largely independent of the size of disturbance (clearing). Lastly, we used PAM fluorometry to show that the photosynthetic condition of Sargassum spp. fronds adjacent to C. filiformis was generally suppressed relative to those distant from C. filiformis. Thus, physical disturbance, combined with invasive traits (e.g. high levels of recruitment and vegetative growth) most likely facilitate the spread of C. filiformis, with the ramifications being lower epibiotic diversity and possibly reduced photosynthetic condition of co-occurring native macrophytes.

Introduction

The spread of introduced invasive plants can have severe impacts on biodiversity [1–3]. Similarly, some native plants are also undergoing range expansions and/or becoming more abundant [4–6]. There is evidence that range expansions and increasing abundances of native species can have ecological effects as great as those of introduced species [7–9]. Although they have received much less attention than their exotic counterparts, the spread of native macrophytes can result in homogenisation of space-limited environments, the creation of space by disturbances that create space can promote the spread of invasive species. For example, the physical removal of native kelp allows colonisation of substrata by the invasive alga Undaria pinnatifida [20]. Colonisation of kelp beds was also dependent on the size of the disturbed patch. In the Mediterranean, anthropogenic disturbances (e.g. nutrients and sediment) negatively affect native kelp, promoting the development of turfing macrophytes which facilitate the colonisation of the invasive green alga C. racemosa [21–23]. C. racemosa does not appear to colonise intact kelp beds [24]. Thus, while the spread of native marine macrophytes...
has been linked to changes in climatic conditions e.g. [5], we may also expect them to spread in areas prone to abiotic disturbance, or where competitors have been removed e.g. [25]. Generally, however, little is known about the conditions that promote the spread of native marine macrophytes.

In New South Wales, Australia, the loss of habitat-forming macrophytes [26] appears to coincide with the spread of the native green alga, *Caulerpa filiformis* (Family Caulerpaceae). *C. filiformis* was first recorded from Botany Bay and Port Jackson [27] although its distribution is considered to be restricted to a 260 km range from Port Stephens to Wollongong [28] (Fig. 1). The supposed proliferation of *C. filiformis* within its range was first noted during the 1970s [29]. More recently, populations have been recorded at locations as far as 350 km north of its previously recorded northern limit (i.e. at Ballina; Glasby unpublished data) (Fig. 1).

The decline of some macrophytes has been attributed to the disturbance effects of urbanisation, such as polluted runoff and historical sewage outfalls [26,30,31], yet species of *Caulerpa* can proliferate under such conditions [29,32]. Thus, *C. filiformis* may be replacing species that are being lost (possibly due to a variety of mechanisms) by occupying newly created space. The spread of *C. filiformis* may have severe implications for the structure and diversity of near-shore coastal communities because *C. filiformis* can form large mono-specific stands (Zhang pers. obs.), is chemically defended and unpalatable to several herbivores, and is structurally simpler than common co-occurring macroalgal species [33]. Structural complexity of a habitat is often positively associated with the diversity of invertebrates e.g. [34,35]. In addition, once established, *C. filiformis* may also affect the health of competitors it interacts with (e.g. via alleopathy, competition for resources, altering abiotic processes; see [3] for review of invasive plant impacts) further aiding its own spread and increasing its impacts.

In this study we investigated the potential mechanisms underpinning the spread of *C. filiformis*. We focused on its interactions with one of the dominant brown algal complexes in the mid to low intertidal, namely *Sargassum* spp. We tested two hypotheses relating to colonization of space created by a disturbance. First that *C. filiformis* would colonize space, via the recruitment of propagules, faster than *Sargassum* spp., regardless of whether that space was created within beds of *Sargassum* spp. (hereafter, *Sargassum* habitat) or within beds of *C. filiformis* (hereafter, *Caulerpa* habitat). Second, in patches created on boundaries between *Sargassum* habitat and *Caulerpa* habitat, *C. filiformis* would colonise the space via vegetative growth faster than *Sargassum* spp. Both of these mechanisms of colonisation can contribute to high demographic rates and the spread of opportunistic species [36]. We also hypothesized that epibiotic assemblages in newly colonized patches in *Caulerpa* habitat would be less diverse than those within *Sargassum* habitat. These three hypotheses were tested by mimicking physical disturbance at multiple sites. We created patches of different sizes because patterns of colonization, and hence assemblage structure, can vary significantly with patch size [37]. We predicted that patch size would influence the magnitude of differences between habitats. Finally, we hypothesized that *C. filiformis* would negatively affect the health of *Sargassum* spp. and tested this by comparing the photosynthetic ability of *Sargassum* spp. where it interacted with *C. filiformis* versus areas where it did not. We tested this latter hypothesis as invasive macrophytes can have sublethal effects on native species that cannot be detected by population level studies e.g. [1,2,38,39].

| Figure 1. Study area along the east coast of Australia, showing 1) sites where sampling and experiments were conducted (Pearl Beach, Cronulla and Bellambi; black triangles), 2) the historical distribution of *Caulerpa filiformis* (Port Stephens to Wollongong; black circles) and 3) Ballina (black star), the site furthest north where *C. filiformis* has been documented. doi:10.1371/journal.pone.0094647.g001 |

| Materials and Methods |

Study species and sites
All sites and organisms sampled were conducted under Permit No. P09/0058-1.0 issued by New South Wales Department of Primary Industries. The field studies did not involve endangered or protected species.

*Caulerpa filiformis* is a green alga found on exposed intertidal and subtidal reefs between 0–6 m water depth in NSW [40] where it grows on a variety of substrata, from rocks to sand [41]. Its root-like rhizomes form dense, entangling mats and give rise to flattened blades with cylindrical, annulated bases. The blades can grow to >40 cm [42]. In NSW, this species is becoming more conspicuous within its range between Port Stephens and Wollongong and is now a dominant intertidal/subtidal habitat-forming species on many rocky shores around Sydney [40,43,44]. Observations suggest that it is now also expanding its geographic range (Zhang and Glasby pers obs).

*Sargassum* is a ubiquitous genus of brown algae in Australian waters [45]. Numerous species have been described and accurate identification is difficult, being based on the size and shape of receptacles [28]. We note that the *Sargassum* spp. at each site appeared morphologically similar and the invasive *S. muticum* has not been recorded in Australia. We use only the generic name here but suggest that the most likely species are *S. linearifolium*, *S. vernacularum*, *S. fallax* or possibly *S. vestitum*.

Clearance experiments were replicated at three sites (Pearl Beach 33°32’56.29”S 151°18’29.54”E, Cronulla 34°04’10.01”S...
Does physical disturbance facilitate the recruitment of propagules of Caulerpa filiformis compared to Sargassum spp.?

To determine whether physical disturbance facilitates the establishment of C. filiformis via recruitment of propagules (i.e. fragments), we created clearings of different sizes (small, 10x10 cm; medium, 20x20 cm; large 40x40 cm) in habitats consisting of Caulerpa habitat or Sargassum habitat in wave-exposed areas on intertidal rocky shores. Recruitment is defined as a recruit that has settled, survived and grown to become visible to an investigator when sampled [48]. Clearings were made in beds of the different macrophytes that were at least 2 m² and at least 1 m apart (rock pools were avoided). The different types of clearings were interspersed to avoid spatially confounding effects, with n = 4 replicates/treatment/habitat.

Clearance treatments were created using paint scrapers to remove all macro-organisms during 22nd to 29th November, 2011. Plots were inspected and groomed fortnightly to prevent vegetative growth of macrophytes into plots. After 4 months, the percent cover was determined, not just cover occupied by the algae, sponges and polychaetes) that had recruited into each patch. Different sized quadrats with different size grids were used to standardize sampling effort within each disturbance treatment (small, 1x1 cm grids; medium, 2x2 cm grids; large, 4x4 cm grids). Sites were sampled in the order that they were established so clearings were in place for the same length of time.

Is the vegetative growth of Caulerpa filiformis into cleared patches higher than for Sargassum spp.?

Here we tested whether high rates of vegetative growth enabled C. filiformis to colonise bare space more rapidly than Sargassum spp. We created the same clearance treatments as described above, but cleared patches were placed on the common boundary between adjacent C. filiformis and Sargassum spp. patches. The experiment was replicated at just two sites (Cronulla and Bellambi). Although the timing of reproduction for Sargassum spp. at our study sites is unknown, Sargassum spp. were common at each site. The experiment on the effects of C. filiformis on the health of Sargassum spp. was replicated at just two sites (Cronulla and Bellambi). The timing of reproduction for Sargassum spp. at our study sites is unknown, Sargassum spp. on the east and south coasts of Australia are reproductive from spring to late summer coinciding with the timing of this study (see below) [46,47].

Is Caulerpa filiformis adversely affecting the health of Sargassum spp.?

Chlorophyll fluorescence measurements change with exposure to stress [49] and can be used to infer changes to the photosynthetic efficiency of a plant [50]. Therefore, we used chlorophyll fluorescence to investigate whether C. filiformis was having negative effects on the health of Sargassum spp. At two sites (Cronulla and Bellambi), we compared chlorophyll fluorescence of Sargassum spp. fronds at the edges of beds that were either in direct contact with C. filiformis or distant from C. filiformis fronds. Sargassum spp. collected from edges not in contact with C. filiformis were at least 1 m away from C. filiformis patches and were often against coralline algae, bare substrata, or the brown alga Hormosira banksii. Sites were sampled on different days in March, 2012, with all replicates from a single site collected and measured (details below) in one day. At each site, a single frond was haphazardly selected at the edge of Sargassum spp. patches adjacent and distant from C. filiformis (n = 15 patches/treatment). Five measures were taken for each frond to account for within frond variation, with the average value being used in analyses.

Measurements of chlorophyll fluorescence

Initially, we measured chlorophyll fluorescence in the field, however the measurements were highly variable (data not shown) - chlorophyll fluorescence can vary greatly temporally and spatially due to the effects of background abiotic conditions and diel responses of the plants [51]. In addition, macrophytes have to be dark-adapted for at least 30 minutes for accurate readings of F0 and Fm' which was difficult to do in the field. Therefore we developed the following standard procedures. Once collected, Sargassum spp. fronds from a single patch (fronds collected adjacent and distant from C. filiformis were kept separate) were placed in a perforated bag and submerged in seawater inside a dark, aerated cooler during transit to the laboratory (max. 2 hrs). All collections took place between 07:00 and 12:00 to minimise diel variation in chlorophyll fluorescence [52]. At the laboratory, fronds were kept separate in clear aerated holding tanks (12x20x15 cm) filled with ~3 cm of filtered (0.2 μm) seawater. Water temperatures in the tanks were maintained between 24–26°C (comparable to field temperatures at time of collection). Using a Diving-PAM (Walz GmbH Effeltrich, Germany: settings; ML int 8, G 12, SP int 12, SP width 0.8s) with a 2 mm plastic fibre, the maximum quantum yield of PSII (Fv/Fm' = (Fm-Fo)/Fm'; where Fm is dark-adapted maximum and F0 is minimum fluorescence) was measured on the dark-adapted samples. The fronds were then light-adapted for 30 min under 400–500 μmol photon m⁻² s⁻¹ irradiance; supplied by 400 W metal halide lamps fitted with a diffuser. Effective quantum yield of PSII (ΦPSII = (Fm'-Fo)/Fm'; where Fm’ is light-adapted maximum and F0 is minimum fluorescence) was estimated using the Diving-PAM. Non-photochemical quenching was determined according to the following equation; NPQ = (Fm-Fm’)/Fm’. NPQ is a response to protect the plants photosystems from excess light energy or environmental stress [53].

Statistical analyses

To create direct tests for differences between the recruitment (via propagules) of C. filiformis and Sargassum spp., we randomly selected two of the four replicate patches to use for each variable (thereby ensuring the data were independent). These data were analysed with orthogonal four factor Analyses of Variance (ANOVA) to determine the effects of patch size (fixed factor with 3 levels; small, medium or large clearings), habitat (fixed factor with 2 levels; Caulerpa or Sargassum habitat), site (random factor
with 3 levels) and a factor termed SvC (i.e., species recruiting, \textit{Sargassum} vs \textit{Caulerpa}, fixed) \((n = 2)\). For all analyses, assumptions of ANOVAs were checked by examining distributions of residuals and plots of residuals vs. means \cite{QuinnKeough2002}. Non-significant interaction terms were pooled with the residual. SNK post-hoc tests were used to test for differences among levels of significant factors.

Because non-significant results for the factor patch in the analysis above \(\text{result not presented}\) may have been due to small samples sizes \((n = 2)\), we conducted separate three factor orthogonal ANOVAs to provide more robust tests of the effects of patch size \(\text{fixed factor with 3 levels; small, medium or large clearings}\), habitat \(\text{fixed factor with 2 levels; \textit{Caulerpa} or \textit{Sargassum} habitat}\) and site \(\text{random factor with 3 levels}\) on the recruitment of \textit{C. filiformis} and \textit{Sargassum} spp. \((n = 4)\). Thus, these analyses did not have a direct comparison of recruitment of \textit{Sargassum} spp. vs recruitment of \textit{C. filiformis}.

Similar to above, a direct test for regrowth of \textit{Sargassum} spp. vs \textit{C. filiformis} was created by randomly selecting two replicates to use for cover estimates of each algal taxon – these data were analysed using a 3 factor orthogonal ANOVA with the factors site \(\text{random factor}\), patch size \(\text{fixed factor with 3 levels; small, medium or large clearings}\) and SvC \(\text{i.e., species colonising, \textit{Sargassum} vs \textit{Caulerpa}, fixed}\). Again, separate orthogonal 2-factor ANOVAs were used to provide more robust tests of the effect of patch size and site on the regrowth of \textit{C. filiformis} and \textit{Sargassum} spp. to cleared patches on the boundary between \textit{Caulerpa} and \textit{Sargassum} habitat.

Epibiotic assemblages \(14 \text{ variables including algae, sessile invertebrates, plus sand and rock}\) that colonized disturbed patches of different sizes were compared between habitats and sites using non-parametric permutational multivariate ANOVA PERMANOVA; \cite{Anderson2001}. The 3-factor design outlined above was used with 9999 permutations of Bray Curtis similarities and Type III sums of

**Figure 2.** Mean percent cover of recruiting propagules \(\pm \text{ SE}\) of \(\text{A) \textit{Sargassum} spp. and B) \textit{Caulerpa filiformis}}\) into small \(S\), medium \(M\) and large \(L\) cleared patches created \(\text{in \textit{Sargassum} habitat (black bars) or \textit{Caulerpa} habitat (grey bars) at three sites (Pearl Beach, Cronulla and Bellambi). Letters indicate results of SNK tests comparing differences in recruitment to \textit{Sargassum} spp. habitat (S) or \textit{Caulerpa} habitat (C) per site (n = 4). doi:10.1371/journal.pone.0094647.g002}}\)
squares. Non-significant interaction terms (P>0.25) were pooled with the Residual to increase the power of tests for other terms in the model. SIMPER was used to identify the variables responsible for differences among factors. Diversity measures (total number of taxa and Shannon diversity index) were compared using the same PERMANOVA design, but based on Euclidean distances among samples.

The effect of C. filiformis on the chlorophyll fluorescence of Sargassum spp. was analysed using orthogonal 2-factor ANOVA with factors site (random factor with two levels), and position (fixed factor with two levels; adjacent to or distant from C. filiformis) with n = 15 replicates/treatment/site. Separate analyses were done for ΦPSII and NPQ. Non-significant interaction terms (P>0.25) were pooled with the Residual.

Results

Does physical disturbance facilitate the recruitment of propagules of Caulerpa filiformis compared to Sargassum spp.?

Direct comparisons of recruitment of C. filiformis vs Sargassum spp. (using n = 2 independent replicates) identified significant differences according to habitat (SvC x Habitat F1,52 = 28.18, P = 0.0001) and Site (SvC x Site F9,52 = 28.18, P = 0.0001). Specifically, recruitment of C. filiformis was significantly greater than recruitment of Sargassum spp. in Caulerpa habitat, and equivalent to Sargassum spp. recruitment in Sargassum habitat. Recruitment of C. filiformis was significantly greater than recruitment of Sargassum spp. at two sites, while there was no significant difference at Pearl Beach (although there was a trend for Sargassum spp. recruitment to be greater at this site (Fig. 2).

Analysed separately, there were interactive effects of Habitat and Site on recruitment of Sargassum spp. (Table 1). Specifically, Sargassum spp. recruitment was greater in Sargassum habitat (mean ± SE, 25±4.2%) than in Caulerpa habitat (1±0.7%) at one site (Pearl Beach), but not significantly different between habitats at the other two sites (where recruitment to each habitat was typically small (6±2%, Fig. 2A). These differences were reflected in the comparison among sites, with recruitment of Sargassum spp. to Sargassum habitat being significantly greater at Pearl Beach than at the other two sites, whereas recruitment of Sargassum spp. to Caulerpa habitat was minimal and did not differ among sites (3±2%, Fig. 2A). Recruitment of Sargassum spp. into medium and large patches was significantly greater than recruitment into small patches (Fig. 2A and next section), with the pattern being consistent among sites and between habitats (Table 1).

The recruitment of C. filiformis also varied interactively by Habitat and Site (Table 2). Specifically, recruitment of C. filiformis was significantly greater in Caulerpa habitat than in Sargassum habitat at two sites, and similar between habitats at the other site (Fig. 2B). At the sites where C. filiformis recruitment was greatest in Caulerpa habitat, its mean percentage cover was 6±2% and 19±3%, while at the site where C. filiformis recruited equally well to both Sargassum and Caulerpa habitats, its mean cover was 27±5%. The recruitment of C. filiformis did not differ significantly among patch size (Table 2).

Is the vegetative growth of Caulerpa filiformis into clearings higher than for Sargassum spp.?

Vegetative growth of C. filiformis into patches was significantly greater than recolonisation of Sargassum spp. (SvC F1,30 = 32.58, P = 0.0001). This result was consistent for all sites and patch sizes. Overall the mean (±SE) percent recolonisation by C. filiformis (25±3%) was three times higher than for Sargassum spp. (8±1%) (Fig. 3). Using the more robust statistical design (with n = 4 replicates) and analysing the two algal taxa separately, no significant differences in recolonisation of either taxon were detected among patch sizes (C. filiformis, F2,24 = 3.08, P = 0.075; Sargassum spp., F2,24 = 2.52, P = 0.095) or among sites (C. filiformis, F2,24 = 0.598, P = 0.556; Sargassum spp., F2,24 = 1.826, P = 0.178).

Are assemblages colonising patches in Caulerpa filiformis different from those in Sargassum spp.?

Epibiotic assemblages that colonised patches after four months differed significantly among patch sizes (Pseudo -F2,64 = 3.49, P = 0.0023). SIMPER analyses showed that small patches were characterised by sand, bare rock and C. filiformis (together comprising 93% of similarity among replicates). In comparison, medium patches had less bare rock, more brown filamentous
alga, coralline algae and Sargassum spp. Large patches had the least bare rock, and the most algae (all species). The average cover of Sargassum spp. was 2% ± 1% in small patches, 12% ± 4% in medium and 12% ± 3% in large patches, whereas C. filiformis cover was consistent among patch sizes (17% ± 6%, 16% ± 4% and 18% ± 4% in small, medium and large patches respectively).

Epibiotic assemblages in patches also varied significantly between habitats at some sites (Habitat x Site Pseudo-F1,56 df = 3.09, P = 0.0047). Pairwise tests indicated that these newly established assemblages differed between habitats at Pearl Beach (t = 3.79, P = 0.0001) and Cronulla (t = 2.14, P = 0.019), but not at Bellambi (t = 1.37, P = 0.135). SIMPER analyses showed that the taxa driving these differences differed between the two sites, so these were investigated with one way univariate comparisons between habitats. Covers of coralline algae were significantly greater in patches created within Sargassum habitat at both Pearl Beach (F1,22 df = 4.44, P = 0.0047) and Cronulla (F1,22 df = 10.03, P = 0.0045). Two other algae, Padina sp. and Laurencia sp. were found only in Sargassum habitat at both sites. The only taxon that was consistently more abundant in patches created in Caulerpa habitat was C. filiformis. At the third site (Bellambi), where newly recruited assemblages did not differ between habitats, all replicate patches were dominated by sand and C. filiformis with the two accounting for ≥85% of similarity among replicates in both habitats.

The percentage cover of sand in patches differed significantly among sites (F2,64 df = 25.16, P = 0.0001; Fig. 4) and was significantly greater in Caulerpa habitat than Sargassum habitat (F1,64 df = 5.22, P = 0.0257). Total number of taxa and Shannon diversity index showed identical patterns among treatments. Shannon diversity increased significantly (Pseudo-F2,66 df = 14.59, P = 0.0001) with patch size (small 0.55< medium 0.76< large 1.04) and was significantly less (Pseudo-F1,66 df = 30.19, P = 0.0001) in patches within Caulerpa habitat (0.58) compared to Sargassum habitat (0.986). These patterns were consistent among sites.

Is Caulerpa filiformis adversely affecting the health of Sargassum spp.?

Photosynthetic activity (ΦPSII) differed according to proximity to Caulerpa habitat at some sites (Position x Site F1,26 = 42.35, P = 0.001). SNK tests showed that ΦPSII of Sargassum spp. was significantly lower where it was adjacent to Caulerpa habitat at Cronulla, but did not differ between positions at Bellambi (although differences were in the same direction as for Cronulla, Fig. 5A). NPQ measurements of Sargassum spp. fronds were significantly higher where they were adjacent to C. filiformis compared to edges against other algae and this was consistent at both sites (F1,12 = 11.94, P = 0.001; Fig. 5B). Lower ΦPSII and higher NPQ indicates the health of Sargassum spp. is reduced in fronds adjacent to Caulerpa habitat.

Discussion

For native species undergoing range expansions or increases in abundance, we may expect positive responses to disturbance. We found support for our hypothesis that physical disturbance (cleared patches created in established assemblages) promotes the recruitment (at two of three sites) and vegetative spread (at all sites) of C. filiformis and, once established, the alga appears to have negative effects on the physiological health of Sargassum spp. In addition, newly colonised patches within Caulerpa habitat supported a less diverse community compared to patches in Sargassum habitat.

Several mechanisms may explain the differences among sites in patterns of recruitment for C. filiformis and Sargassum spp. In coastal ecosystems, increasing sedimentation can alter macrophyte community structure by removing dominant habitat-forming macrophytes and inhibiting their recruitment success, as well as
facilitating sediment tolerant species [55–60]. In our study, the percentage cover of sand in cleared patches was lowest at Pearl Beach (sand was absent from patches), the site of highest Sargassum spp. recruitment and lowest C. filiformis recruitment, intermediate at Cronulla and highest at Bellambi where the recruitment of C. filiformis was highest and Sargassum spp. very low (Fig. 4). Caulerpa spp. may have been more abundant at sandy sites due to greater tolerance to sedimentation than Sargassum spp. [61,62], although some species of Sargassum are capable of recruiting to cobbles covered with fine sediment [63]. Macroalgae that are able to trap and bind sediments can benefit from sedimentation [55,58]. Piazzi et al. [22] showed that, on rocky shores, the invasive alga, C. racemosa, was not affected by sedimentation, whereas several native macrophytes were hindered by sediment deposition. Thus, increasing sedimentation may promote the establishment of C. filiformis. Indeed, this species has been documented as being positively associated with sand in Australia [29] and South Africa [64]. It is possible that sediment deposition may be an important mechanism of disturbance that is creating space for C. filiformis, perhaps in addition to nutrient enrichment as proposed by [29]. In addition, the limited recruitment of C. filiformis at Pearl Beach may reflect a lesser propagule supply. Propagule pressure is often a strong driver of the spread of invasive species and also for some marine macrophytes undergoing range expansions, particularly in disturbed environments [25,65]. Although we did not quantify it in this study, the sizes of intertidal and subtidal populations of C. filiformis were smallest at Pearl Beach and largest at Bellambi. The intertidal Caulerpa habitat at Pearl Beach was restricted to one large patch while the alga was spread widely across the intertidal rock platform at Bellambi, with a more intermediate distribution at Cronulla. Populations of C. filiformis may need to reach a threshold size before propagule supply is large enough to create a positive feedback and accelerated expansion of the population [66].

Habitat surrounding cleared patches (either Sargassum or Caulerpa) was an important mediator of the recruitment of Sargassum spp. and C. filiformis. This may relate simply to proximity of adult algae, or may reflect each habitat’s ability to trap sediment (possibly promoted by the dense longer fronds of C. filiformis compared to Sargassum spp. in this study). Secondary metabolites from C. filiformis could also have hindered recruitment of Sargassum spp. to Caulerpa habitat, as grazers may differentially consume Sargassum spp. recruits over C. filiformis [67,68]. It is likely that the habitat surrounding cleared patches also affected the diversity of epibiota associated with newly recruited patches, which was always greater within Sargassum habitat than within Caulerpa habitat. This pattern held even at Bellambi where cleared patches within Sargassum habitat were actually colonized by ~28% C. filiformis, which was greater than within Caulerpa habitat (20% C. filiformis colonization). That is, the significantly greater diversity in the patches within Sargassum habitat was most likely due to the surrounding habitat rather than the dominant alga that colonized the patches at this site. Caulerpa habitat may be less diverse than Sargassum habitat due to the reduced structural complexity of the former, the greater percentage of sand and/or sediment anoxia (and accumulation of toxic sulphides) which negatively affect biota associated with species of Caulerpa [39,69].

Recruitment of algae also varied with level of disturbance (i.e. cleared patch size); small clearances contained lower proportions of Sargassum spp. and brown filamentous algae compared to medium and large clearances. Our findings differ from Airoldi [70] who showed that Sargassum spp. tended to recruit more to smaller compared to larger cleared patches (although results were
not significant). The apparent discrepancy in results most likely relates to the difference in patch sizes used in the two studies; Airoldi [70] used patches (150–320 cm²) that were intermediate between our small (100 cm²) and medium (400 cm²) patch sizes. Moreover, recruitment by *Sargassum* spp. in our study (5–35%) was far greater in all patch sizes than found by Airoldi (1–10%).

Vegetative growth into cleared patches on the boundary between habitats was consistently higher for *C. filiformis* compared to *Sargassum* spp. across all sites and disturbance levels. This suggests that, once established, *C. filiformis* is generally better able to exploit freshly disturbed space via vegetative growth than *Sargassum* spp. This may be because *Sargassum* spp. were more damaged by the process of clearing space and/or because *C. filiformis* can grow faster. The latter explanation is likely as species of *Caulerpa* are known to grow very quickly from all parts stems, leaves and roots; [71]. Fast vegetative growth is a trait typical of opportunistic species [36] and appears to be common for macrophytes in impacted sediments [see 61 for review]. In addition, vegetative growth likely further stabilises sediments facilitating *C. filiformis’ own growth and giving it a competitive advantage over *Sargassum* spp. However, the germinating zygotes of species of *Sargassum* are retained on the fronds before being

**Figure 5. Mean (±SE) phytosynthetic activity of *Sargassum* spp. fronds measured as (A) ΦPSII or (B) NPQ at two sites.** Measurements (n = 15 fronds/edge/site) were taken at edges of *Sargassum* habitat which were either adjacent to *Caulerpa* habitat (black bars) or and away from (grey bars). *above bars indicate significant results of SNK comparisons of means.

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released with a sticky rhizoid which can facilitate quick (and nearby) attachment e.g. [72].

One aspect we did not address was temporal variation in colonisation of cleared patches. Kennelly [73] found that removal of kelp, leaving only the understorey, resulted in cleared patches colonised by turf except in winter, when kelp recruitment was high and better able to recolonise space. Although the timing of Sargassum spp. reproduction does vary temporally and spatially [74], in our study C. filiformis was able to colonise Sargassum habitat at two sites during a period when Sargassum spp. were recruiting to cleared patches (i.e. Sargassum spp. were reproductively active). This suggests that Sargassum spp. may generally be poor competitors for space, which is consistent with Airoldi's findings over 12 months [68]. However, at our Pearl Beach site, where C. filiformis did not recruit to Sargassum habitat, Sargassum recruitment into medium and large patches (34±2.8%) was comparable to the recruitment of C. filiformis to Caulerpa habitat (29.3±4.7%) and Sargassum habitat (27.9±9.9%) at Bellambi. Thus, at some sites (and perhaps times of the year), Sargassum spp. may outcompete C. filiformis for space, particularly where there is less sediment or a smaller population of C. filiformis (e.g. Pearl Beach). Importantly, the loss of habitat-forming macrophytes and associated communities can persist years after disturbance [75]. For example, removal of Ascophyllum nodosum canopy (i.e. leaving the understory in place) resulted in colonisation by two species of Fucus for 12 years [76,77]. Understanding seasonal variation in recruitment dynamics to disturbed patches, and the temporal response of C. filiformis, Sargassum spp. and the communities they support post-colonisation, will be an important avenue for future research.

Whilst disturbance is an important mechanism facilitating the initial establishment of opportunistic macrophytes, once established, some can successfully outcompete native species – i.e. they can switch from being passengers to drivers of ecological change [24,78,79]. Similarly, in this study we found some evidence for negative effects of the native C. filiformis on the physiological health of Sargassum spp. This could have resulted from several mechanisms. First, C. filiformis could overshadow Sargassum spp. (Caulerpa fronds can reach >40 cm) reducing light levels and limiting its access to essential elements from the water column [80]. However, this seems unlikely because Sargassum spp. fronds were typically of a comparable height to C. filiformis. Moreover, NPQ usually decreases when macrophytes are shaded as the xanthophyll cycle relaxes [81], yet we measured an increase in NPQ of Sargassum spp. fronds that were against Caulerpa. Second, C. filiformis may alter the abiotic conditions of the trapped sediments by inducing sediment anoxia - dense mats of macrophyte species can cause substrate anoxia via a reduction in photosynthesis, increased algal respiration and detritus accumulation [39,82,83] - and/or producing toxic sulphides to which it is tolerant [84]. Third, production of allelochemicals that negatively affect competitors can promote the spread of some macrophytes [85,86]. Although secondary metabolites (e.g. Caulerpenyne) from Caulerpa spp., including C. filiformis are unpalatable to most grazers [33,87], it is not known whether these or other potential allelochemicals could affect Sargassum spp.. Understanding the mechanisms by which C. filiformis is potentially affecting Sargassum spp. and the demographic consequences for Sargassum spp. warrants further investigation.

Here we have shown that physical disturbance (creation of space) can enhance the recruitment and promote vegetative growth of a native alga, C. filiformis, whose potential increase in abundance and spread may have serious consequences for coastal biodiversity. This study was conducted on intertidal rock platforms where Sargassum spp. appear to be the main competitors with C. filiformis. C. filiformis is more common on subtidal reefs (Glasyb, unpubl data), where it also co-exists with species of kelp (e.g. Ecklonia radiata) and other brown algae (e.g. Phyllospora comosa) at several sites throughout its distribution (Gribben, Glasyb pers obs). Many of these subtidal brown macrophytes share similar reproductive strategies to Sargassum spp. [80]. Thus, following physical disturbance these subtidal habitats may also be susceptible to colonisation by C. filiformis. Indeed, C. filiformis appears to be replacing these important subtidal habitat-forming macrophytes at several sites throughout its distribution (Gribben, Glasyb pers obs). Given growing coastal populations, and predicted increases in physical disturbance in coastal ecosystems (e.g. increased frequency and intensity of storms), these attributes may facilitate an increase in abundance and/or spread of species such as C. filiformis. However, further research is required to incorporate broader temporal scales into understanding the consequences of its interactions with native macrophytes and communities more generally.

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Author Contributions

Conceived and designed the experiments: DZ TMG PJR PEG. Performed the experiments: DZ. Analyzed the data: DZ TMG PEG. Contributed reagents/materials/analysis tools: PJR PEG. Wrote the paper: DZ TMG PEG.

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