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RESEARCH ARTICLE

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Megaherbivores may impact expansion of invasive seagrass in the Caribbean

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Abstract

1. Our knowledge of the functional role of large herbivores is rapidly expanding, and the impact of grazing on species coexistence and nonnative species expansion has been studied across ecosystems. However, experimental data on large grazer impacts on plant invasion in aquatic ecosystems are lacking.
2. Since its introduction in 2002, the seagrass species *Halophila stipulacea* has rapidly expanded across the Eastern Caribbean, forming dense meadows in green turtle (*Chelonia mydas*)—foraging areas. We investigate the changes in seagrass species coexistence and the impacts of leaf grazing by green turtles on nonnative seagrass expansion in Lac Bay (Bonaire, Caribbean Netherlands).
3. Green turtle grazing behaviour changed after the introduction of nonnative seagrass to Lac Bay in 2010. Field observations, together with time-lapse satellite images over the last four decades, showed initiation of new grazing patches (65 ha, an increase of 72%). The sharp border between grazed and ungrazed seagrass patches moved in the direction of shallower areas with native seagrass species that had previously (1970–2010) been ungrazed. Green turtles deployed with Fastloc-GPS transmitters confirmed high site fidelity to these newly cropped patches. In addition, cafeteria experiments indicated selective grazing by green turtles on native species. These native seagrass species had significantly higher nutritional values compared to the nonnative species. In parallel, exclosure experiments showed that nonnative seagrass expanded more rapidly in grazed canopies compared to ungrazed canopies. Finally, in 6 years from 2011 to 2017, *H. stipulacea* underwent a significant expansion, invading 20–49 fixed monitoring locations

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in Lac Bay, increasing from 6% to 20% in total occurrence. During the same period, native seagrass *Thalassia testudinum* occurrence decreased by 33%.

4. *Synthesis*. Our results provide first-time evidence of large-scale replacement of native seagrasses by rapidly colonizing *Halophila stipulacea* in the Caribbean and add a mechanistic explanation for this invasiveness. We conclude that green turtle leaf grazing may modify the rate and spatial extent of this invasive species' expansion, due to grazing preferences, and increased space for settlement. This work shows how large herbivores play an important but unrecognized role in species coexistence and plant invasions of aquatic ecosystems.

KEYWORDS

alien invasive species, *Chelonia mydas*, exotic, foundation species, *Halophila stipulacea*, landscape modification, plant–herbivore interactions, *Thalassia testudinum*

1 | INTRODUCTION

Large herbivores, whether aquatic or terrestrial, can have strong impacts on associated species and can be critical ecosystem engineers as they alter plant productivity, modify geomorphology, and influence nutrient cycling, habitat structure and plant coexistence (Bakker et al., 2016; Poore et al., 2012; Wood, Armstron, & Richards, 1967). Megaherbivores can impact plant species coexistence and species composition via multiple mechanisms. Preferential grazing on dominant plant species can promote species diversity by releasing competitors (Olff & Ritchie, 1998), or reduce diversity by selectively removing nondominant species (Hidding, Bakker, et al., 2010). Grazing can also precipitate species shift from long-lived, slow growing species, to faster growing pioneer species that are better adapted to grazing (Kelkar, Arthur, Marba, & Alcoverro, 2013; Knapp et al., 1999). Additionally, grazing on below-ground plant parts can enhance species diversity by creating regeneration niches through sediment disturbance (Hidding, Nolet, Boer, Vries, & Klaassen, 2010).

The impact of herbivory on plant coexistence has been shown across diverse ecosystems, including examples from terrestrial and aquatic systems (Augustine & McNaughton, 1998; Bakker, Pagès, Arthur, & Alcoverro, 2015). This being the case, there is a potential for grazers to increase the success of invasive plants. Evidence of interactions between grazing and invasive plants go both ways. Nonnative species may come to dominate by escaping specialist consumers (enemy release hypothesis; Keane & Crawley, 2002). Elsewhere, grazers exert significant regulation of introduced species abundance as nonnative species are maladapted to deter herbivores (biotic resistance hypothesis; Levine, Adler, & Yelenik, 2004; Parker & Hay, 2005; Parker, Burkepile, & Hay, 2006). Evidence of interactive effects between grazing and invasive plants are less well studied in aquatic systems compared to terrestrial systems. In aquatic systems, small grazers can impact plant invasions (Valentine & Johnson, 2005). However, the impacts of grazing by megaherbivores on introduced aquatic macrophytes have not been empirically investigated.

Following its recent introduction, the seagrass species *Halophila stipulacea*, native to the Red Sea, has spread rapidly throughout the Caribbean (Willette et al., 2014). Compared to other introduced algae and seagrasses (Williams, 2007; Williams & Smith, 2007), *H. stipulacea* has demonstrated an exceptional ecological flexibility in salinity, depth, habitat, and light requirements (Willette et al., 2014). Given this flexibility, supplemented by its clonal expansion, the nonnative *H. stipulacea* has spread rapidly from island to island (Willette et al., 2014). The first records of *H. stipulacea* out-competing native species (Maréchal, Meesters, Vedie, & Hellio, 2013; Steiner & Willette, 2015) suggest that *H. stipulacea* will quickly become abundant at the expense of native seagrasses. This invasion has the potential for far-reaching ecological and economic impacts and therefore, *H. stipulacea* is described as "invasive" in the Caribbean (Rogers, Willette, & Miller, 2014; Willette et al., 2014). However, no large-scale replacement or harm to native species has been observed so far.

To date, no mechanistic approach has been undertaken to investigate competition between *H. stipulacea* and native seagrasses. The mechanisms aiding the expansion of *H. stipulacea* are not fully resolved, since experimental evidence on species interactions, with both competitors and herbivores, is lacking (Rogers et al., 2014; Smulders, Vonk, Engel, & Christianen, 2017; van Tussenbroek et al., 2016), making the potential impacts of the *H. stipulacea* invasion difficult to predict. Large grazers such as green sea turtles (*Chelonia mydas*) may influence the responses of native seagrasses to introduced species settlement and expansion. A recent increase in global sea turtle populations (Chaloupka et al., 2008) is returning more seagrass areas to a naturally grazed state, however, the impact of grazing on (invasive-) species coexistence has not been adequately considered so far.

In the Caribbean, green sea turtles typically consume large amounts of turtle grass (*Thalassia testudinum*, henceforth referred to as "native" seagrass). Grazing patches in *Thalassia* meadows are easily recognized, as turtles crop seagrass leaves in specific patches or zones that they maintain and revisit to stimulate the production of new, highly nutritious leaves (Hernandez & van Tussenbroek,

2014; Preen, 1995), comparable to "grazing lawns" in terrestrial systems. Turtle grazing further results in shorter leaves, lower shoot density, and lower below-ground biomass (Christianen et al., 2012). Therefore, we hypothesize that turtle grazing may impact species coexistence and invasive seagrass expansion via selective grazing of native seagrasses, their historically preferred food source, and by releasing space for subsequent settlement by opening the canopy when cropping.

In this study, we investigated the expansion of *H. stipulacea*, species coexistence between native and introduced seagrasses, and the impacts of grazing by green turtles on nonnative seagrass expansion in Lac Bay, Bonaire, Caribbean Netherlands. Our aims were addressed in a six-step approach: we (i) mapped recent and historic locations of turtle grazing patches by comparing a time series of satellite images, and (ii) determined current feeding hotspots (grazing locations) of green turtles in the bay by deploying satellite trackers and field observations. Then, we (iii) experimentally assessed turtle food preference for native and introduced seagrass species, compared (iv) the nutritional content of invasive and native seagrass species, and (v) quantified the colonization rates of invasive seagrass in native species meadows with (the exclusion of) green turtle grazing. Furthermore, the changes in meadow composition in the 6 years since *H. stipulacea* introduction in Lac Bay were (vi) mapped using monitoring data on the occurrence of invasive and native seagrass. Finally, we discussed the implications of our results and the role of megaherbivores on species coexistence and plant invasions of aquatic ecosystems under the anticipated global change in large grazer populations and species introductions.

2 | MATERIALS AND METHODS

2.1 | Study area

The study area was Lac Bay, Bonaire (Caribbean Netherlands). Lac Bay is a shallow inland lagoon, of approximately 7 km^2 with a maximum depth of 6 m, located at the windward eastern coast of Bonaire, Caribbean Netherlands (12°06'N 068°14'W, Figure 1a). The average annual rainfall is low (463 mm/year) and the tidal range is limited (30 cm; Freitas, Nijhof, Rojer, & Debrot, 2005). The area is a Ramsar site (Wetlands International, 2017) due to its high natural value and important ecosystem services. The bay supports high levels of biodiversity by providing key habitats for water birds (Debrot, Bemmelen, & Ligon, 2014), fish, and invertebrates (Hylkema, Vogelaar, Meesters, Nagelkerken, & Debrot, 2014; Nagelkerken et al., 2002), including the endangered Caribbean queen conch (*Lobatus gigas*; Engel, 2008).

The east side of the bay is protected from wave action by a fringing reef. The bay is connected to the sea by a deep-water channel at its northernmost tip through which turtles access the bay. The density of grazing green turtles is high and Lac Bay is a year-round key foraging area for turtles from rookeries across the wider Caribbean (Debrot et al., 2012). Contrasting to some regional and long-term trends, the first investigation of recent monitoring data of Lac Bay (2005–2016) did not show a significant increase in green turtle abundance (Table S1, Figure S1, Sea Turtle Conservation Bonaire, 2012, 2016). Although there has not been a significant increase in number of turtles in the past 10 years, the densities of the foraging aggregations in our study area appear to be on the high side when compared regionally (Debrot et al., 2012). Red mangroves (*Rhizophora mangle*) border and encroach the north and west side of the bay (Debrot et al., 2012; Erdman & Scheffers, 2006). Seagrasses and macro-algae cover most of the bay mainly dominated by the native species *T. testudinum* (Figure 1d) and *Syringodium filiforme*, and the nonnative *H. stipulacea* (Figure 1e), along with beds of the calcareous alga *Halimeda* spp. The bay contains ~200 hectares of seagrass, and is 1 of the 20 sites in the Caribbean Sea where *H. stipulacea* has been reported (Willette et al., 2014). Recent monitoring in Lac Bay showed rapid expansion of *H. stipulacea* at a local scale during a 4-year period (Smulders et al., 2017). Lac Bay thus provided a unique opportunity to study interactions between introduced plants and megaherbivores.

2.2 | Location of recent and historic green turtle grazing patches

Temporal changes in cropped locations within the bay (termed "grazing patches") were estimated using a time series of satellite images (from 1970 to 2016). We drew benthic maps and outlined the border between ungrazed and grazed patches during multiple years; both from before *H. stipulacea* invasion (1970, 2006, 2010) and after the *H. stipulacea* invasion (2012, 2014, 2016). Ungrazed *T. testudinum* meadows were visible on satellite images as a darker underwater zone lining the mangrove area (Figure 1b). Grazed patches were visible on satellite images as a lighter area below the border of this darker zone. The resulting line polygons were confirmed in the field in 2016 by two observers; one snorkelling and the other kayaking while mapping the border using a handheld GPS. The distance between the border between ungrazed and grazed areas in 2010 and 2016 was estimated as the shortest distance between the lines at 20 random points. The area between the two lines was estimated using the area calculator tool in QGIS.

2.3 | Green turtle movement patterns

Grazing behaviour by green turtles on native and introduced seagrass species was assessed by determining foraging patterns and feeding preferences. Current foraging hotspots for green turtles were identified and compared to seagrass meadow composition in Lac Bay. We deployed Fastloc-GPS transmitters (SPLASH10-F-351A, Wildlife computers, USA) that collected highly accurate location data from six green turtles (curved carapace length 67, 70, 73, 82, 82, and 83 cm respectively) over an average period of 88 (± 19) days between July–November 2015 and October 2016–March 2017. Turtles were caught with nets or hand captured in Lac Bay, and subsequently released at the position of capture. When captured, all six turtles were seen to have seagrass leaf remains (*T. testudinum*) inside their mouths on visual

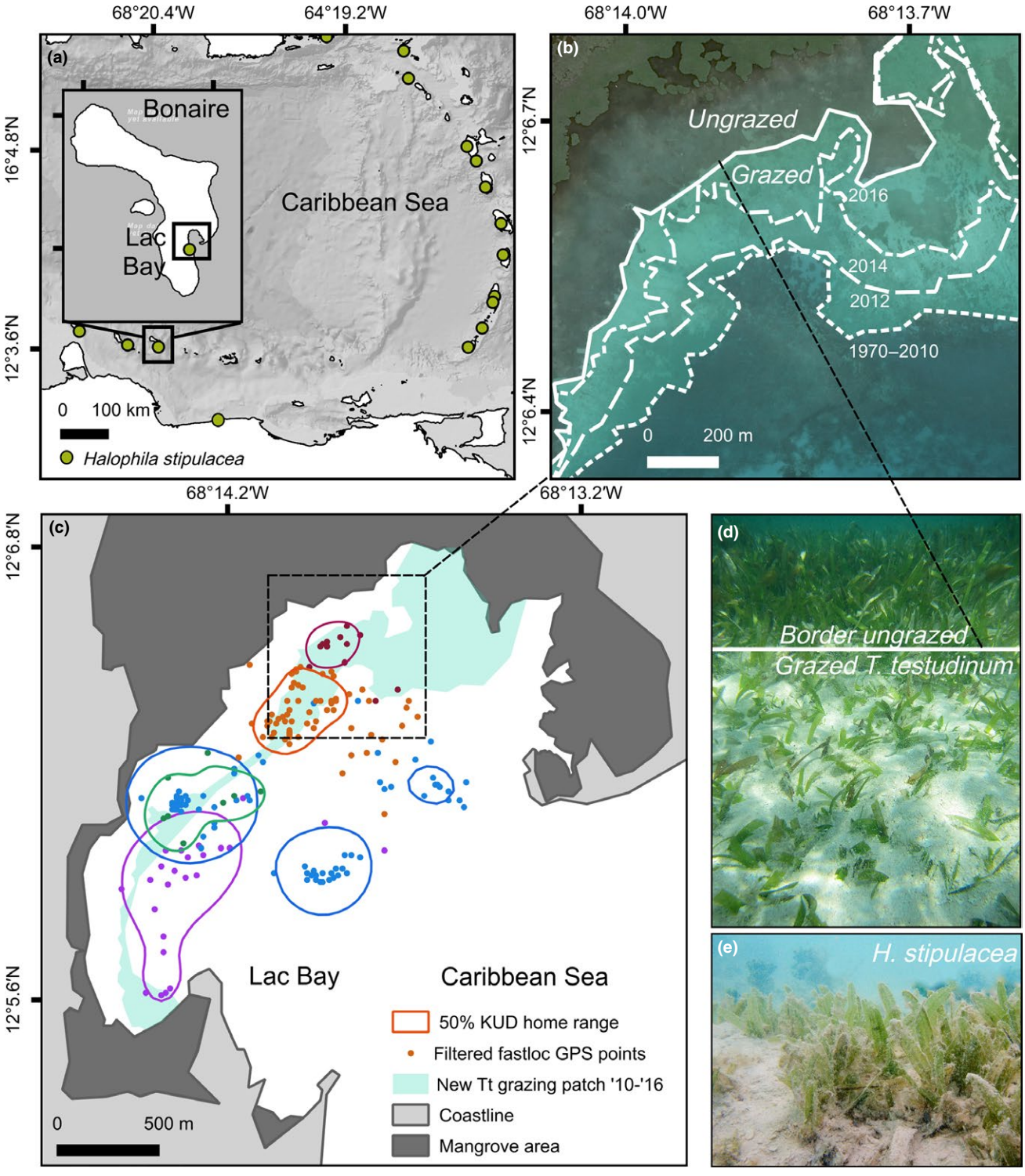

FIGURE 1 (a) The location of Bonaire, study site Lac Bay (inset), and the geographical distribution of *Halophila stipulacea* along 16 Eastern Caribbean islands where *H. stipulacea* has been recently reported (modified from Willette et al., 2014 and Vera et al., 2014). (b) Aerial picture of the north-east section of Lac Bay with drawn lines showing the shifting border between grazed (darker) and ungrazed (lighter) *Thalassia testudinum* (Tt) over multiple years; before *H. stipulacea* invasion (January 1970, 2010), and after *H. stipulacea* invasion (February 2012, 2014 and 2016). The border moves towards the shallower area bordering the mangroves (top left). The area between outer lines represents the same "new grazed patches" as in figure panel (c) and is presented as a filled blue polygon. Aerial picture: Google earth 2016. (d) Native *T. testudinum* with the typical sharp border between ungrazed (top) and grazed (bottom) patches and (e) invasive seagrass *H. stipulacea*. (c) Foraging hotspots (50% kernel utilization distribution (KUD) home range, line polygons) of five green turtles tracked in 2015 and 2017 concentrate in the area where new cropping (or "grazing") patches have been initiated in previously ungrazed *T. testudinum* area (filled blue polygon). Points present the filtered turtle locations for five colour-marked individuals (with unique PTT ID nr's): orange 151,225, red 151,221, green 151,222, blue 162,896, purple 162,897. The inset shows the outline of figure panel (b). Photo (d) and (e) by MJAC [Colour figure can be viewed at wileyonlinelibrary.com]

inspection. After attachment of satellite transmitters, locations were received from Argos via the Wildlife computers' data portal. We used Fastloc-GPS locations derived from four to nine satellites. Prior to the data analysis, we plotted all locations to visually identify outlying data points representing likely erroneous locations (e.g., located on land) and we followed previously established standard methods to exclude likely erroneous Fastloc-GPS locations using the following steps (Christiansen, Esteban, Mortimer, Dujon, & Hays, 2017; Dujon, Lindstrom, & Hays, 2014; Hays et al., 2014; Luschi, Hays, DelSeppia, Marsh, & Papi, 1998; Thomson et al., 2017). Firstly, we excluded all locations with a residual ≥35 and we assessed if locations were biologically feasible based upon known green turtle swimming speeds (no more than 200 km/day assuming 24 hr travel (Dujon et al., 2014). Further visual examinations of plotted tracks were used to identify when the turtles had departed from their foraging ground (e.g., for long-distance migration). At this point, the turtles would travel in a single persistent direction as opposed to swimming back and forth within a relatively restricted area (Christiansen et al. 2017). All location data collected after the time of departure were excluded from analyses. In order to avoid pseudo replication, we only retained one randomly selected location per day (Christiansen et al. 2017). Finally, we selected all locations that were recorded on seagrass habitat, inside Lac Bay. Green turtle home range sizes were estimated using Kernel Utility Distribution (KUD, Worton, 1989) as implemented in the adehabitatHR package (Calenge, 2006) in R (R Core Team, 2017), using the reference bandwidth (h_{ref}) as the smoothing parameter (extent = 0.2, grid = 100; Thomson et al., 2017). Activity centres (foraging hotspots) were identified using 50% KUD (Worton, 1989, Christiansen et al. 2017), and mapped using QGIS.

2.4 | Green turtle foraging preferences

Green turtle seagrass species preferences were determined by cafeteria (or food choice) experiments (Becking, Bussel, Debrot, & Christianen, 2014). A total of 59 cafeteria experiments were undertaken in Lac Bay between October–December 2013, July–November 2015, and October–December 2016. In order to account for the previously observed high site fidelity of green turtles, the setup was deployed at multiple sites within Lac Bay, differing in seagrass assemblages (dominated by *T. testudinum* or by *H. stipulacea*) at a water depth between 1.7 and 4.0 m. The setup consisted of three seagrass tethers, each with a bundle of leaves of similar size from one of the three locally dominant seagrass species (*T. testudinum*, *S. filiforme*, and *H. stipulacea*), placed in random order at each deployment (Figure 2). Tethers were attached on top of rebar sticks (30 cm high, 1.2 cm diameter) using cable ties, and spaced by 0.5 m. A GOPRO camera (Hero 3 with attached battery BacPac, GoPro Inc. USA) was placed at a distance of 2 m from the tethers and recorded unattended for 2–4 hr. The number of grazing events was recorded from the video footage. The number of grazing events was defined as the number of individual turtles that physically grazed on seagrass material from the tethers.

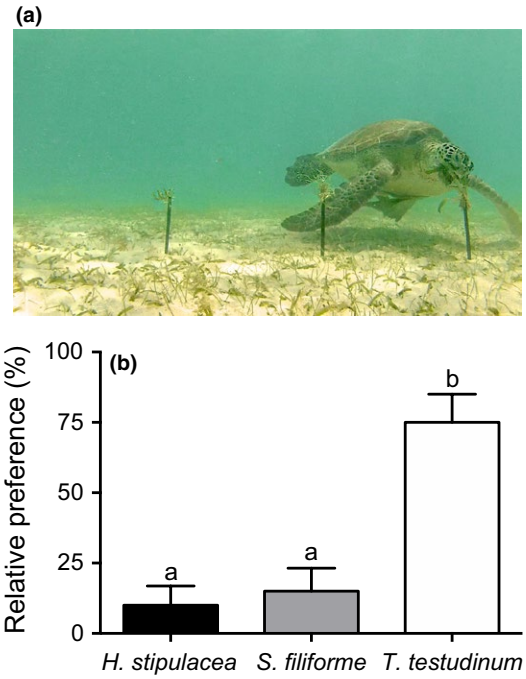

FIGURE 2 (a) Setup of a "cafeteria" (or food choice) experiment for green turtles in Lac Bay, Bonaire, (b) here native seagrass *Thalassia testudinum* is preferred above invasive *Halophila stipulacea*. The relative number of grazing events that a species was eaten, *n* = 20, Friedman's test. Photo by MJAC [Colour figure can be viewed at wileyonlinelibrary.com]

2.5 | Comparison of nutritional content between native and introduced seagrass species

We compared native (*T. testudinum* and *S. filiforme*) and introduced (*H. stipulacea*) seagrass species biomass and leaf nutritional content. Seagrass samples were collected at 12 locations across Lac Bay where there were clear indications of green turtle grazing. At each site, species were sampled using a core (15.3 cm diameter, 20 cm deep). Sediment was removed, leaves were cleaned of epiphytes and all material was rinsed with water, dried for 48 hr at 60°C, and the biomass of all plant parts was measured separately per species. Dried leaves were ground using pestle and mortar, then approximately 8 mg of homogenized material was used to determine leaf carbon and nitrogen content with a carbon–nitrogen–sulphur analyser (Vario ISOTOPE cube; Elementar, Germany). To ensure technical reproducibility we performed triplicate measurements from each of the 12 replicate samples for each species. Leaf phosphorus content was determined from 150 mg homogenized dry plant material, which was digested with 4 ml HNO_3 (65%), 2 ml HCl (37%) and 1 ml H_2O (100%), using a microwave lab station (Multiwave sample preparation system, Perkin-Elmer-Anton Paar physica, Austria). We analysed six replicates per species. Digestions were diluted, and the concentration of phosphorus determined with an ICP Spectrometer (Optima 8000 ICP-OES, Perkin-Elmer, MA, USA). Soluble sugar content was determined from 7 mg dry plant material extracted in 80% ethanol in 12 replicates per species. Starch was subsequently

extracted from the ethanol-insoluble fraction by hydrolysis in 3% HCl and boiled at 100°C for 30 min. Soluble sugars and starch extractions were measured in an anthrone assay standardized to sucrose (Yemm & Willis, 1954). Light absorption was measured on a plate reader at 625 nm (SPECTROstar Nano, BMG LABTECH, Germany). All samples were measured in duplicate and a calibration curve was prepared for every series of measurements (soluble sugar, starch).

2.6 | Impacts of grazing on expansion of invasive species

To assess the impact of turtle leaf grazing on plant competition through clonal expansion by *H. stipulacea*, we measured the development of *H. stipulacea* cover in 1.5 by 1.5 m plots with and without natural leaf grazing by green turtles during 4.5 months (July–November 2015). These plots were placed at random in selected locations in the seagrass meadow at similar depths and initially contained no *H. stipulacea*. The cover of *H. stipulacea* was monitored within a 25 by 25 cm frame in the middle of the plots after 12, 47, 60, 74, 89, 103, and 134 days respectively. The impact of natural grazing was assessed from five plots marked in a naturally grazed meadow using four galvanized steel pins protruding 10 cm above the sediment surface. To create plots without grazing, we employed five turtle exclusion cages (l × w × h: 1.5 × 1.5 × 0.3 m) constructed of galvanized steel mesh (15 × 15 cm, 0.9 cm diameter wires). The mesh excluded sea turtles but permitted passage of smaller bodied animals (e.g., fish) and ensured a negligible impact on light transmission to the seagrass bed (Christianen et al., 2012). The vertical sides of the cages were extended into the sediment to prevent entry of large animals. The cages were accessed by observers through the top. Algae growth on the cage mesh was minimal during the experiment and algae were actively cleaned off the cages every 2 weeks.

2.7 | Changes in seagrass occurrence since introduction of *H. stipulacea*

In order to map recent changes in species occurrence for *H. stipulacea* and the native seagrass species in Lac Bay, we quantified seagrass occurrence in 2011 (the year after the first reported occurrence of *H. stipulacea* (Willette et al., 2014) and in 2017. Seagrass occurrence was determined at 49 fixed monitoring locations spaced evenly at intervals of 250 m. The position of each location was estimated using a handheld GPS (eTrex 10, Garmin) after which six replicated 1-m^2 quadrats were assessed. The presence of *T. testudinum*, *H. stipulacea*, and *S. filiforme* was assessed and counted in 100 equal squares within the 1-m^2 quadrat by two independent observers. The average of the six replicated 1 m^2 measurements was taken as the measure of relative occurrence per sampling location. This relative occurrence of each seagrass was plotted in QGIS (Quantum GIS Development Team, 2017).

2.8 | Statistical analysis

Prior to model fitting, all data were checked for normality using Shapiro–Wilks tests ($p = 0.05$) and further confirmation by visual validation of the final models. If the normality assumption was not met, data were transformed. All relevant transformations are mentioned in the figures or table legends. The multiple-choice feeding assays were analysed with a nonparametric Friedman's test and a post hoc Friedman Nemenyi test (Roa, 1992). The differences in plant nutritional value characteristics were analysed with an ANOVA with seagrass assemblage as a factor. Regression slopes for the development of *H. stipulacea* with and without grazing were compared using an ANCOVA with grazing as a factor and time as a continuous covariable. Statistics were performed in R (R Core Team 2017). Average values are presented together with standard errors (*SE*).

3 | RESULTS

3.1 | Changes in turtle grazing patches

During 2010 and 2016, the border between ungrazed and grazed *T. testudinum* moved towards the shallower areas by 146 ± 21.2 m along the northern mangrove fringed border of Lac Bay (Figure 1b). In contrast, the border did not move during the period from 1970 to 2010, that is, before the introduction of *H. stipulacea* to Lac Bay. The total area of ungrazed *T. testudinum* decreased by 64.9 hectares while the total area that was grazed increased to 155 hectares during the period from 2010 to 2016. The grazed area covered 78% of the total area of seagrass habitat (~200 ha) that was present at the research site in 2016.

3.2 | Green turtle foraging patterns and preferences

Green turtles deployed with Fastloc-GPS transmitters confirmed high site fidelity to these newly grazed patches. Five of the green turtles that were deployed with transmitters generally foraged on the seagrass meadows inside Lac Bay, while one individual migrated to Venezuela immediately after it was tagged (latter not included in analysis). The filtering of the Fastloc-GPS-transmitted data (as described in the methods) resulted in the removal of 381 locations from a total of 1848 locations. The green turtles restricted their movements to relatively small areas, identified from 50% Kernel Utility Distribution (KUD) (Figure 1c). We refer below to these areas as "foraging hotspots." Most individual sea turtles focused at sites with a single centre of activity; only one individual moved regularly between three foraging hotspots (turtle ID 162896, Figure 1c). These restricted movements indicated a high degree of site fidelity for each turtle within the seagrass meadows. The locations of the foraging hotspots of five tracked turtles overlapped the area where new grazing patches were initiated in areas previously occupied by ungrazed *T. testudinum* (depicted by the blue polygon in Figure 1b,c). The foraging activity seemed to be centred in areas with the highest occurrence of *T. testudinum* and at the border between ungrazed and grazed *T. testudinum* mapped in 2016 (Figure 1b,c).

From the cafeteria experiments we found that green turtles appeared to prefer consuming tethered *T. testudinum* (Figure 2b) over *H. stipulacea* or *S. filiforme*. We have repeated the cafeteria experiment 59 times, however, we did not record a turtle on the video during each deployment. In total, 365 turtles were observed (0–10 m from camera) and 20 grazing events were recorded. There was a significant difference in feeding preference of green turtles between seagrass species ($p < 0.001$, $F = 15.7$, Figure 2). Two turtles grazed on *H. stipulacea*, three turtles grazed on *S. filiforme*, and 15 turtles grazed on *T. testudinum*. We recorded grazing events only when the experimental setup was placed within a grazed *T. testudinum* assemblage (34 times deployed), not when the setup was placed within a *H. stipulacea* assemblage (25 times deployed). Video footage of grazing events indicated that individual green turtles visually inspected seagrass tethers and skipped bundles of *H. stipulacea* and *S. filiforme* before grazing on tethered *T. testudinum* (Appendix S1, video of green turtle selectively grazing on native seagrass tethers). Green turtles were the only large herbivores in this system, the density of mesoherbivores (e.g., herbivorous fish and urchins) was very low in at the experimental sites (pers. obs. MJAC and FOHS).

3.3 | Comparing seagrass nutritional content

The comparison of native and introduced seagrass in grazed meadows in Lac Bay revealed that the grazed leaf biomass was similar for both *T. testudinum* (44.83 ± 17.50 g DW m^{-2}) and introduced *H. stipulacea* (54.60 ± 9.76 g DW m^{-2}, Figure 3g), while the grazed leaf biomass was significantly lower (ANOVA, $p = 0.024$) for *S. filiforme* (22.41 ± 11.67 g DW m^{-2}). The nutritional values were significantly higher for leaf material collected from the native *T. testudinum* compared to the invasive *H. stipulacea* and the other native *S. filiforme* seagrass. Nitrogen and phosphorus content were significantly higher, and C:N ratios were significantly lower for *T. testudinum* ($p < 0.001$; Figure 3a,c,e) compared to *H. stipulacea*. Two types of soluble carbohydrate were tested: the soluble sugars content in *T. testudinum* leaves was significantly higher ($p = 0.016$, Figure 3b) compared to *H. stipulacea* and *S. filiforme* leaves, whereas we detected no statistical difference in the starch content ($p = 0.86$, Figure 3d). The leaf soluble sugars content and leaf N content per square meter was 1.8 times higher and 1.7 times higher, respectively, in grazed *T. testudinum* compared to *H. stipulacea*.

3.4 | Impacts of grazing on clonal expansion of *H. stipulacea*

Leaf grazing of *T. testudinum* by green turtles significantly impacted clonal expansion rate of *H. stipulacea* in native meadows (Figure 4). After 134 days, *H. stipulacea* appeared in three of five grazed plots with an average cover of 10.0% ± 4.9%, and in one of five ungrazed plots, with an average occurrence of 1.0% ± 1.0%. The initiation of clonal expansion of *H. stipulacea* was faster in grazed plots (first reported at 12 days) compared to ungrazed plots (first reported at 103 days). The increase in *H. stipulacea* occurrence after 134 days was significantly different between grazed and ungrazed plots ($F = 19.84$, $p < 0.001$). At the end of the experiment, *T. testudinum* cover was significantly lower in plots that had been

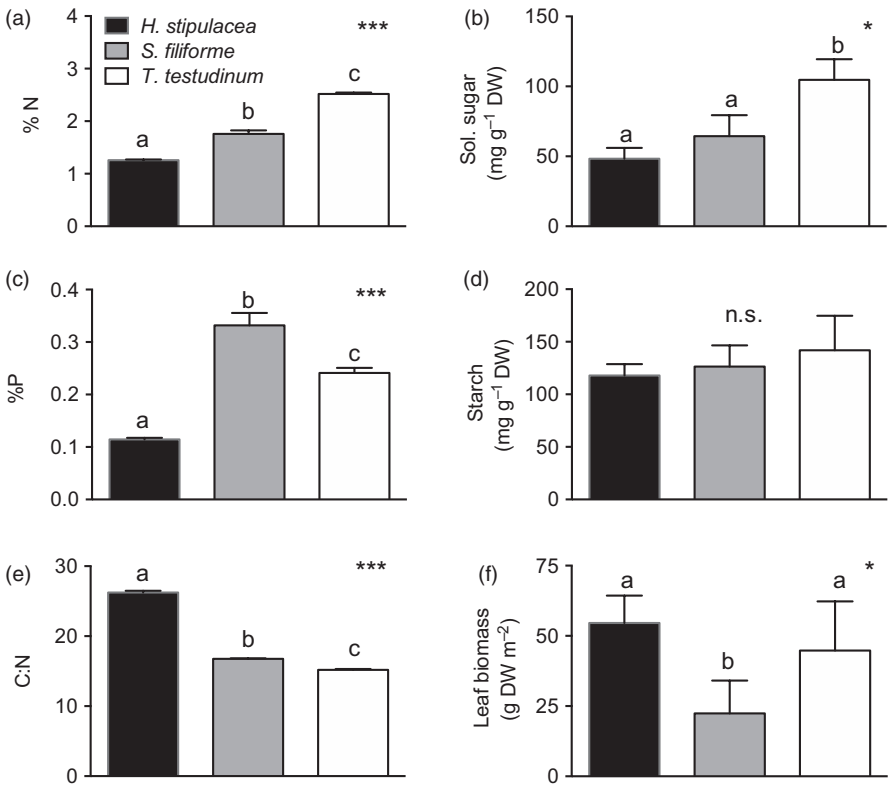

FIGURE 3 Comparison of leaf material of the invasive *Halophila stipulacea* (Hs), *Syringodium filiforme* (Sf), and *Thalassia testudinum* (Tt) in the grazed area of Lac Bay, in; (a) nitrogen content ($n = 36$), (b) soluble sugar content ($n = 14$), (c) phosphorus content ($n = 7$), (d) starch content ($n = 14$), (e) C:N ratios ($n = 36$), and (f) leaf biomass ($n = 36$). Significant differences are shown by different letters *$0.01 \le p \le 0.05$, ***$p < 0.001$. Average values are presented together with standard errors (*SE*)

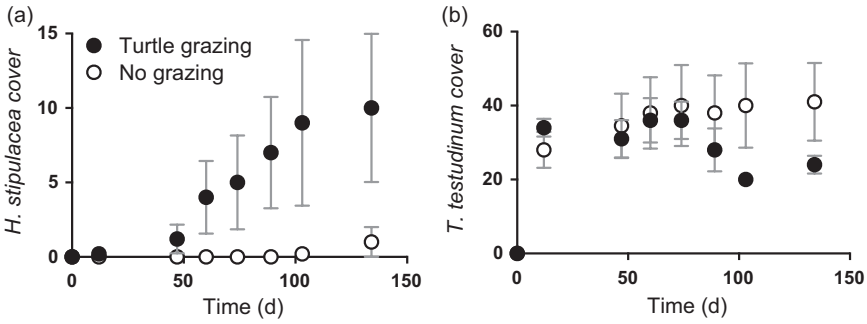

FIGURE 4 Effect of turtle grazing on colonization rate of (a) invasive species *Halophila stipulacea* within native *Thalassia testudinum* dominated meadows and (b) native species *T. testudinum*, $n = 5$. Average values are presented together with standard errors (*SE*)

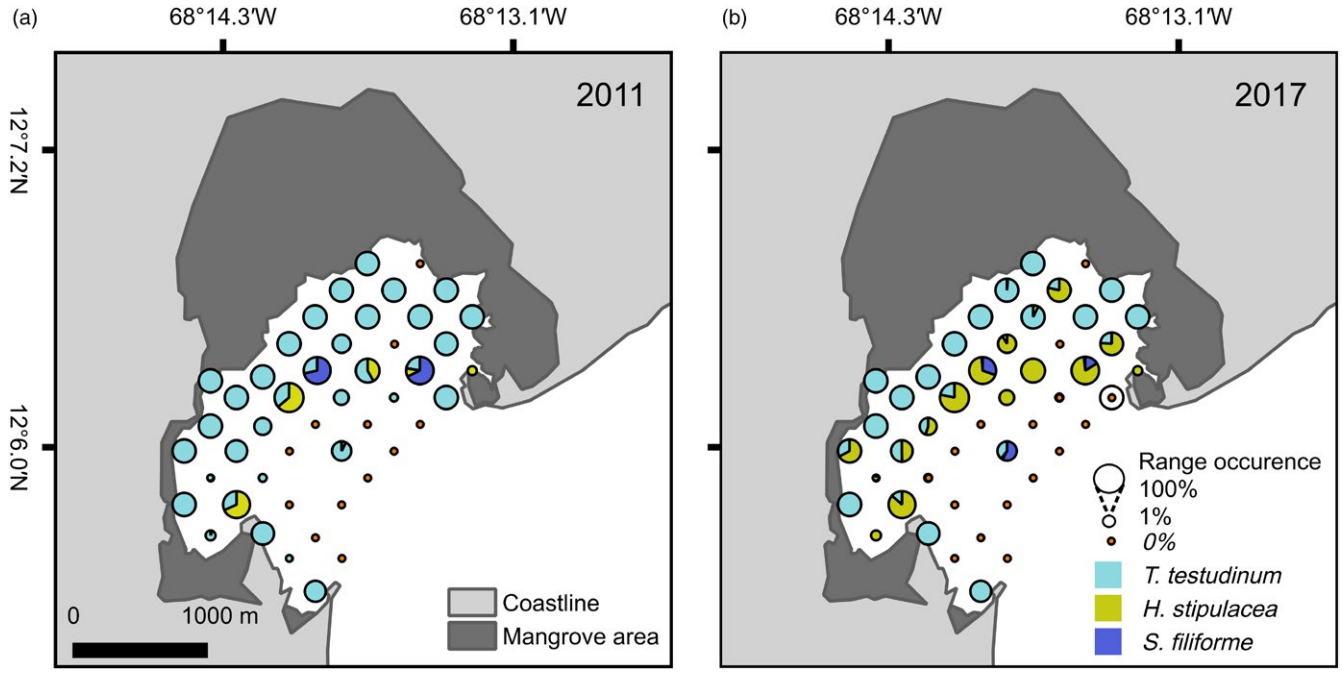

FIGURE 5 The relative occurrence per seagrass species (a) in 2011 and (b) in 2017. Forty-nine fixed monitoring locations were spaced evenly in intervals at 250 m in Lac Bay, Bonaire. Pie charts are scaled to absolute total seagrass occurrence for each monitoring location [Colour figure can be viewed at wileyonlinelibrary.com]

colonized by *H. stipulacea* (24% ± 2.5%) compared to uncolonized plots (41% ± 10.5%; $F = 13.43$, $p < 0.001$). However, the change in *T. testudinum* occurrence over time was not significantly different between grazed and ungrazed plots ($p > 0.05$).

3.5 | Changes in seagrass occurrence

Overall, we observed an increase in seagrass occurrence from 60.1% to 63.2% in Lac Bay during the period from 2011 to 2017. The occurrence of the invasive seagrass *H. stipulacea* increased from 5.5% ± 2.8% occurrence in 2011 to 25.8% ± 5.8% occurrence in 2017 ($p < 0.001$), whereas the occurrence of the native *T. testudinum* decreased from 50.8% ± 6.1% in 2011 to 34.2% ± 6.0% in 2017 ($p < 0.001$). We failed to detect a significant change in occurrence of the native *S. filiforme*, which was detected at 3.8% ± 2.7% occurrence in 2011, and at 3.2% ± 2.2% occurrence in 2017 ($p = 0.82$). In 2011, *H. stipulacea* was observed at six locations in the deeper, central area of Lac Bay and spread to 20 new fixed monitoring locations in more shallow areas of Lac Bay within 6 years (Figure 5). By 2017, *T. testudinum* disappeared from six locations while the occurrence of *H. stipulacea* increased. Near the mangrove border, *T. testudinum* was still the dominant seagrass in 2017, with an occurrence at the fixed sampling locations directly adjacent to the mangroves at >90%. However, visual observation in areas between sampling locations, confirmed the occurrence of *H. stipulacea* in ungrazed, dense *T. testudinum* meadows at depths up to 0.2 m.

4 | DISCUSSION

Using a combination of long-term monitoring and remote sensing of seagrass habitat, telemetry of herbivores, and field caging experiments, we found strong evidence that green turtle leaf grazing may increase the rate and spatial extent of invasive seagrass *H. stipulacea* expansion in the Caribbean. Indirect effects of grazing on species invasions (i.e., apparent competition) have been considered elsewhere (Enge, Nylund, & Pavia, 2013; Orrock, Baskett, & Holt, 2010). This prior work has focused mostly on small mesograzers. Thus, our work

suggests that large herbivores can also trigger the expansion of invasive species by suppressing native species which may be higher in palatability but competitively inferior. Large herbivores play an important but yet largely unrecognized role in invasions of aquatic ecosystems, however, adequate consideration of their impacts is getting increasingly important (Bakker et al., 2015), especially with the anticipated global change in species invasions and large grazer populations (e.g., trophic downgrading, Estes et al., 2011; marine defaunation, McCauley et a., 2015). Our results provide important insights into the degree of species coexistence of native seagrass with invasive seagrasses and show that large herbivores can have an important role in the expansion of the invasive species.

Prior to this study, invasive seagrass expansion has been linked to many factors, but not to grazing. Expansion rates were reported to be high due to high productivity (Smulders et al., 2017), and preferentially occurring in more sheltered (Steiner & Willette, 2015) and euthrophied sites (van Tussenbroek et al., 2016). Many other factors may be involved, including high fragment viability (>2 weeks; Smulders et al., 2017), a potential high seed dispersal distance through megaherbivores (<650 km as found with *Halophila* spp. seeds in Australia; Tol et al., 2017), and the impact of disturbance on fragment density (grazing roots up fragments; Smulders et al., 2017). However, so far, seed dispersal may not be significant as only sterile (Willette et al., 2014) or male plants (Vera, Collado-Vides, Moreno, & Tussenbroek, 2014) have been found in the Caribbean. The rapid expansion of *H. stipulacea* is not a local phenomenon (Willette et al., 2014). Therefore, a combined assessment of the multiple mechanisms and parameterization of these factors is needed to model the future expansion of this species throughout the Caribbean.

A striking result was that since 2010, the year of the introduction of the nonnative seagrass *H. stipulacea*, the sharp border of grazed and ungrazed native seagrass patches moved towards shallower areas. These areas contained native seagrass species that had previously been ungrazed, encompassing a surface area of 65 hectares. The grazing border had previously remained at a stable location based on satellite images ranging as far back as 1970, also during the turtle population increase in the last decades. When food supply (native seagrass) was still high in other areas of the bay, turtles did not prefer to graze in the shallow depths of these meadows, presumably because green turtles experience difficulties attaining neutral buoyancy in shallow depths (Hays, Metcalfe, & Walne, 2004). Faced with increased intraspecific competition for resources at our study site, and in light of their strong preference for the declining native seagrass, we hypothesize that sea turtles shifted to graze beyond this border and expanded their foraging areas into shallow regions of the bay, leading to increased space for settlement and spread of introduced seagrass species.

Following their severe historical depletion due to overharvest, sea turtles have been noticeably increasing in density in the leeward Dutch Caribbean islands, including Bonaire, in recent decades, most likely thanks to increased protection (Debrot, Esteban, Scao, Caballero, & Hoetjes, 2005). However, in contrast to regional long-term trends, green turtle abundance did not increase significantly in the period just before and during the expansion of invasive seagrass in Lac Bay (2005–2016) (Table S1, Figure S1, Sea Turtle Conservation Bonaire, 2012, 2016). Thus, the decline in native seagrasses does not appear to be predominantly fuelled by the increased grazing pressure of turtles, as their population growth rate was not significant and did not match the rate of spread of the invasive seagrass in the area. The observed nonlinear response of declining native seagrass to grazing and invasive species supports the notion that positive feedback mechanisms play a role. Initially, turtle grazing increased the suitability for rapid settlement and the expansion of invasive species. Once the invasive species had settled, intraspecific competition for space between seagrass species may have adversely affected the expansion of native seagrass. This forced the turtles to shift to graze elsewhere and to clearing of native seagrass areas in previously untouched areas thus fuelling further expansion of invasive species. With the projected increase in population density of these megaherbivores (Chaloupka et al., 2008; Mazaris, Schofield, Gkazinou, Almpanidou, & Hays, 2017), and a declining foraging habitat that is often ignored in conservation strategies, the invasion of nonnative seagrass may be accelerated as we here have described and measured.

The interactive effects between megaherbivores and invasive seagrass may impact seagrass species coexistence and species competition. Green turtles are described to have a foraging preference for seagrass species with the highest palatability and nutrient content (Bjorndal, 1997) which are characteristics attributed to fast-growing species (such as *H. wrightii*) over slower growing species (such as *T. testudinum*; Christianen, 2013). The invasive *H. stipulacea* seems to be an exception to this rule. Although it is a fast-growing species, the relative nitrogen content of *H. stipulacea* (a proxy for palatability or nutritional value) and sugar content is almost twice as low as observed in the slower growing native species. Together with the reported grazing preferences, the low leaf nitrogen content may help to explain why green turtles seem to limit invasive *H. stipulacea* as a food source so far. Our results follow the "enemy release hypothesis" (ERH), where "invasive species can become much more dominant as they escape from grazers that are maladapted to eat non-native species" (Keane & Crawley, 2002). Thus, the grazing preference of turtles for more highly nutritious native species can facilitate invasive seagrass expansion.

Although herbivore food preferences are informative, these preferences can change over time (Trowbridge, 1995), induced by both plants and grazers. Plants can respond in time by allocating more chemical deterrents (Wikstrom, Steinarsdottir, Kautsky, & Pavia, 2006). Since few chemical deterrents have been observed in seagrasses (Olsen et al., 2016), their impact on changing preferences is expected to be limited. Turtle food preferences and foraging behaviour may also change in the future since large changes in seagrass cover of native (–20% cover in 6 years) and invasive seagrass species (+14% cover) were observed at our study site in Bonaire. This may result in lower plant–herbivore encounter rates (Parker & Hay, 2005), eventually forcing turtles to switch to nonnative food sources or migrate to alternative foraging areas.

Although *H. stipulacea* was already often considered to be invasive, we provide first-time evidence for the replacement of native seagrass species by an invasive seagrass species, including a mechanistic explanation for this invasiveness. Taken together, our results support labelling *H. stipulacea* as invasive species to the Caribbean area. In contrast to previous reports that only found dense invasive seagrass mats at high environmental nutrient concentrations (van Tussenbroek et al., 2016), we also found dense *H. stipulacea* mats in noneutrophied areas. Together with our results on turtle impacts, this highlights that the "invasiveness" of this species is not only be driven by abiotic environmental conditions (e.g., van Tussenbroek et al., 2016). Our experiments and observations clearly showed that the invasive seagrass is competitively inferior to the native at this location, as shown by limited expansion in ungrazed plots, and requires grazing or other disturbances to establish and spread. The time-series data also indicate that the invader is advancing through space and time in concert with grazing (Figures 1b and 5). However, under undisturbed conditions, and at longer time-scales, it is less clear whether *H. stipulacea* can actively push out native seagrass species. So far, shallow-rooted invasive *H. stipulacea* was only reported to rapidly displace shallow-rooted *S. filiforme* and *H. decipiens* in the Caribbean (Steiner & Willette, 2015; Willette & Ambrose, 2009, 2012 ; Willette et al., 2014). Here, we report that invasive seagrass mats are replacing deeper rooted *T. testudinum*. This can potentially compromise the ecosystem services of the seagrass meadow. For example, a decrease in root biomass may lead to decreased carbon sequestration (Marba et al., 2015), and a decreased stabilization of the seafloor during storms and thus decreased coastal protection (Christianen et al., 2013; Vonk, Christianen, Stapel, & O'Brien, 2015).

The invasion of this nonnative seagrass may not only have important consequences for the carrying capacity of seagrass meadows for green turtle populations but also on green turtle health and growth rates. Under continued expansion of invasive seagrasses and replacement of more nutritious native seagrass by invasive seagrass, turtles may need a larger foraging area of this lower quality food source to meet their daily nutritional needs. This only holds if the area of seagrass foraging habitat is limited and alternative foraging grounds are difficult to find. Seagrass meadows are rapidly being lost (Waycott et al., 2009), specifically in the heavily developed coastal areas of the Caribbean (van Tussenbroek et al., 2016). If turtles are unable to adapt to the new species composition by adjusting their foraging strategy, this could ultimately result in overall decreased turtle growth rates (Bjorndal et al., 2017) and health within the Caribbean region. Our research highlights the need to consider adequate and appropriate foraging and breeding habitat when trying to conserve or protect sea turtles.

5 | SUMMARY: INVASIVE SPECIES EXPANSION AND MEGAHERBIVORES

Based on our results we summarize here how megaherbivore grazing may impact invasive plant expansion using seagrass ecosystems and green turtles as a model (graphical abstract). In tropical seagrass ecosystems, herbivory can facilitate invasive species expansion by a hypothetical positive feedback mechanism. Green turtles selectively graze on native seagrass species *T. testudinum* (happy emoticon; Figure 2) that have higher nutritional value (Figure 3) and rarely choose to eat invasive seagrass (sad emoticon) with a less nutritious foraging area as a result. By leaf cropping, turtles open up the leaf canopy (i.e., shorter leaves, lower shoot density), which was found to facilitate the expansion of invasive seagrass (thicker arrow) (Figure 4). As the biomass of native seagrass species gets scarcer, turtles search for new local grazing locations with native seagrass and initiate grazing patches in shallower areas that were previously ungrazed (Figure 1), triggering accelerated expansion of the invasive seagrasses into these newly grazed shallow areas (Figure 5) and accelerated replacement of native seagrasses.

We conclude that grazing by megaherbivores may modify the rate and spatial extent of the expansion of invasive seagrass species, due to grazing preferences and by increasing space for settlement. The anticipated expansion of invasive seagrass combined with observed increases in green turtle populations and a global decline in seagrass habitat warrants future investigations of interactions between grazing and invasive species expansion in relation to the resilience and recovery of seagrass meadows, seagrass ecosystem services, and sea turtle populations. This work shows how large herbivores play an important but unrecognized role in species coexistence and plant invasions of aquatic ecosystems.

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AUTHOR CONTRIBUTIONS

M.J.A.C. led the writing of the manuscript and conceived the ideas; M.J.A.C., J.A.V., and L.E.B. designed methodology; M.J.A.C., F.O.H.S., M.I.N., and M.S.E. collected the data; M.J.A.C. and F.O.H.S., analysed the data and M.J.A.C., L.E.B., M.I.N., A.O.D., and P.J.P. acquired funding for the project. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

Data archived in 4TU.ResearchData: https://doi.org/10.4121/uuid:772a6bcf-983d-4be5-96bf-ba6175df5634 (Christianen et al., 2018)

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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