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Short Communication

# Non-native seagrass *Halophila stipulacea* forms dense mats under eutrophic conditions in the Caribbean

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## ABSTRACT

Seagrasses comprise 78 species and are rarely invasive. But the seagrass *Halophila stipulacea*, firstly recorded in the Caribbean in the year 2002, has spread quickly throughout the region. Previous works have described this species as invasive in the Caribbean, forming dense mats that exclude native seagrass species. During a reconnaissance field survey of Caribbean seagrass meadows at the islands of Bonaire and Sint Maarten in 2013, we observed that this species was only extremely dense at 5 out of 10 studied meadows. Compared to areas with sparse growth of *H. stipulacea*, these dense meadows showed consistently higher nutrient concentrations, as indicated by higher leaf tissue N contents of the seagrass *Thalassia testudinum* (dense when $C:N < 22.5$) and sediments (dense when $\%N > 11.3$). Thus, the potential invasiveness of this non-native seagrass most likely depends on the environmental conditions, especially the nutrient concentrations.

## 1. Introduction

Human activities have greatly aided long-distance dispersal of many species, and are responsible for the introduction of non-native marine macrophytes throughout the globe. The most common vectors are unintentional introduction by aquaculture and fouling of the hulls of marine vessels and yachts. Several non-native seaweeds are invasive and have profound negative effects in their introduced range (reviews Williams and Smith, 2007; Inderjit et al., 2006), but much less is known about the effects of non-native seagrasses, the other group of marine macrophytes.

There are only few records of seagrass species outside their native range; being *Halophila stipulacea* (origin: Indian Ocean and Red sea, non-native: Mediterranean and Caribbean; Lipkin, 1975; Ruiz and Ballantine, 2004), *Zostera japonica* (origin: temperate and subtropical Western Pacific, non-native: temperate eastern Pacific, Harrison and Bigley 1982) and *Halophila ovalis* (origin: tropical Indo Pacific, non-native: Antigua in the tropical Atlantic, Short et al., 2010). The most well-known is *H. stipulacea*, a small dioecious tropical seagrass that immigrated likely through the Suez Canal into the eastern Mediterranean Sea (Lipkin, 1975). Its first established populations were reported in the 1920s, after which *H. stipulacea* slowly expanded westward (Lipkin, 1975). In 2006, it was found as far as Salerno, western Italy (Gambi et al., 2008). *H. stipulacea* is a fast-growing species tolerant to a wide range of environmental conditions (Lipkin, 1975; Jacobs and Dicks, 1985; Malm, 2006). Boudouresque and Verlaque (2002) considered *H. stipulacea* as one of the nine invasive species among 85 introduced macrophyte species in the Mediterranean, but without referring to any report where this species was considered ecologically or economically harmful. Duarte (2000) stated that the introduction of *H. stipulacea* in the Mediterranean had not caused any reported damage to the local seagrass meadows.

Ruiz and Ballantine (2004) reported *H. stipulacea* for the first time in the Caribbean Sea at the island Granada in 2002. Since then, this species has expanded its distribution to many Caribbean coasts (Willette et al., 2014; Vera et al., 2014). Its introduction into the tropical Atlantic has probably occurred by yachts (Ruiz and Ballantine, 2004), which may also be responsible for its rapid spread throughout the Caribbean Sea (Willette et al., 2014; Vera et al., 2014). Until date only sterile (Willette et al., 2014) or male plants (Vera et al., 2014) have been found in the Caribbean. DeBrot et al. (2011); Willette and Ambrose (2009, 2012) and Willette et al. (2014) described this non-native seagrass as invasive in the Caribbean, referring to its rapid expansion throughout the Caribbean and its potential to form dense mats that exclude native seagrasses.

But, non-native species are not necessarily invasive. According to the Convention of Biological Diversity, we define invasive alien (or non-native) species as species whose introduction and/or spread outside their natural past or present distribution threaten biological diversity

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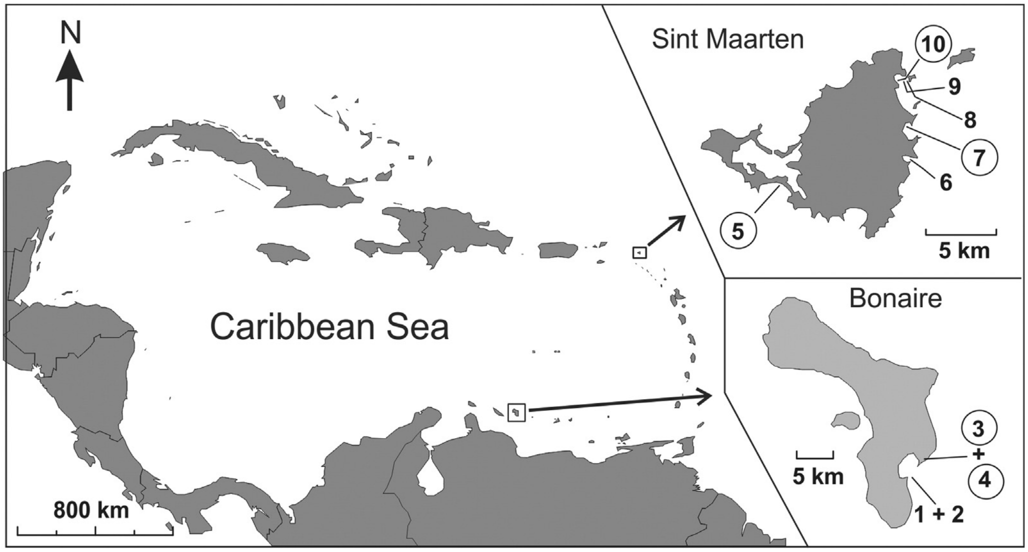

**Fig. 1.** Map of sites at Bonaire and Sint Maarten. 1) Lac Bay-site 1A; 2) Lac Bay-site 1B; 3) Lac Bay-site 2A; 4) Lac Bay-site 2B; 5) Simpson Bay; 6) Oyster Point ; 7) Baie L'Embouchure; 8) Pinel-site 1; 9) Pinel-site 2; 10) Pinel-site 3. The sites in circles had dense *Halophila stipulacea*.

(Verbrugge et al. submitted for publication). During a pilot field campaign visiting ten Caribbean seagrass meadows at the islands of Bonaire and Sint Maarten in May 2013, we observed the non-native *H. stipulacea* formed dense mats at some, but not all sites. We hypothesized that this dense growth form may be related to environmental conditions, especially nutrient availability and sediment characteristics.

## 2. Materials and methods

Ten sites at the islands Bonaire and Sint Maarten were visited between 24th May and 3rd June 2013 (Fig. 1). The depths of these sites varied between 0.5–3.0 m, and we sampled in areas where *H. stipulacea* was present, and registered whether other seagrasses were present (Table 1). At each site, we sampled 3–5 foliar shoots of *Thalassia testudinum* for leaf tissue analysis, because the leaf N and P contents of this species reflect sediment nutrient condition (Fourqurean et al., 1992; Govers et al., 2014). In addition, two sediment samples were taken nearby the seagrass sampling points, pushing a 20 ml syringe into the sediment until ~10 cm depth.

Seagrass foliar samples were collected for analysis of nutrients, sulfur and possible metal contaminants. Calcareous epiphytes were carefully removed with a scalpel, and the leaves were subsequently dried at 60 °C for 48 h. Dried samples were ground and %C and % N were determined with an elemental analyzer (Type NA 1500 Carlo Erba, Thermo Fisher Scientific Inc., USA), coupled online to a mass-spectrophotometer (Thermo Finniga DeltaPlus, USA). Total leaf phosphorous, sulfur and metal concentrations were determined after digestion of dried plant material with nitric acid and $H_2O_2$ by inductively coupled plasma emission spectrophotometry (IRIS Inteprid II, Thermo Electron Corporation, Franklin, MA, USA). Standard references (IPE-858, IPE-137; WEPAL, the Netherlands) were included in the analysis and the average deviation amounted to 3%.

Sediment samples were also dried after sampling (60 °C, 48 h) and sieved (1 mm) to remove large debris. Next, sediment grain size of this material was determined by laser diffraction on a Malvern (Master 2000) particle size analyzer. Sediment organic matter content was determined by weight loss on ignition (LOI) at 550 °C. For element analysis (%N, %C), the sieved and dried sediment was ground and then analyzed in the same way as leaf samples.

The potential factors influencing displayed growth forms (sparse or dense) of *H. stipulacea* were explored using a TREE analysis in R v. 2.15.3. Decision trees are exploratory analyses that construct classification trees based on boolean choices. This TREE analysis identified the variables, and their splits, which best predicted the (dense or sparse) growth forms of *H. stipulacea*. We realized two separate analyses: the first for the plant characteristics and the second for the sediment characteristics using overall mean values for the stations. Pearson correlation coefficients were determined between plant or sediment characteristics that explained best the growth form of *H. stipulacea*, and other measured variables.

## 3. Results

During our surveys, we noted that *H. stipulacea* displayed two distinct growth forms: 1) sparse (low shoot density with bare sand visible among the shoots, as an understory species or at the periphery of mixed

**Table 1**
Description of the sampling sites.

| Site no | Site | Coordinates | Depth (m) | Seagrass species | Growth form *H. stipulacea* |
|---|---|---|---|---|---|
| 1 | Lac Bay 1A | 12°05′37″N 68°14′06″W | 0.3–0.5 | *T. testudinum*, *S. filiforme*, *H. wrightii*, *H. stipulacea* | Sparse |
| 2 | Lac Bay 1B | 12°05′37″N 68°14′06″W | 0.3–0.5 | *T. testudinum*, *H. stipulacea* | Sparse |
| 3 | Lac Bay 2A | 12°06′20″N 68°13′21″W | 2.5 | *T. testudinum*, *H. stipulacea* | Dense |
| 4 | Lac Bay 2B | 12°06′20″N 68°13′21″W | 0.5–1.0 | *T. testudinum*, *H. stipulacea* | Dense |
| 5 | Simpson Bay | 18°02′47″N 63°05′52″W | 1.0 | *T. testudinum*, *H. stipulacea* | Dense |
| 6 | Oyster Point | 18°03′12″N 63°00′57″W | 0.2–0.3 | *H. stipulacea* | Sparse |
| 7 | Baie L'Embouchure | 18°04′19″N 63°01′03″W | 2.0–3.0 | *T. testudinum*, *S. filiforme*, *H. wrightii*, *H. stipulacea* | Dense |
| 8 | Pinel 1 | 18°06′24″N 63°01′02″W | 2.0 | *T. testudinum*, *S. filiforme*, *H. wrightii* | Sparse |
| 9 | Pinel 2 | 18°06′12″N 63°01′12″W | 1.5 | *T. testudinum*, *H. stipulacea* | Sparse |
| 10 | Pinel 3 | 18°06′12″N 63°01′21″W | 2.0–2.5 | *H. stipulacea* | Dense |

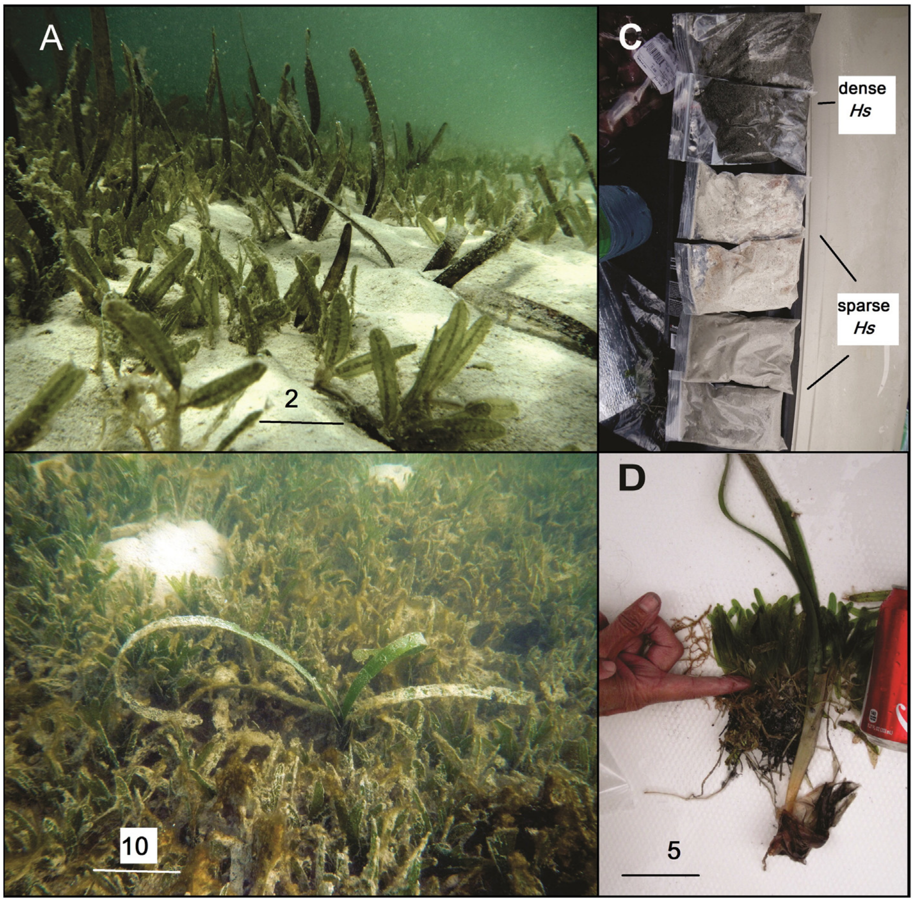

**Fig. 2.** Illustrations of *Halophila stipulacea*. A. Sparse growth at Bonaire-Lac Bay-site, growing at the edge of a dense meadow dominated by *Thalassia testudinum* (larger upright leaves in the back-ground), B. Dense at Bonaire-Lac Bay-site 2: the smaller seagrass is *H. stipulacea* with a single large foliar shoot of *T. testudinum*, C. Sediment samples from beds at Pinel 1–3 (from left to right); note black color indicating anoxia when *H. stipulacea* was dense, D. Rooting depths of dense *H. stipulacea* and native *T. testudinum*: the finger points at the sediment level (note anoxic sediment attached to the seagrasses). Hs *Halophila stipulacea.*

seagrass beds (Fig. 2A), and 2) dense, forming a thick continuous seagrass mat (Fig. 2B).

The TREE analysis detected an unequivocal relation between the growth form of *H. stipulacea* (sparse or dense) and the nitrogen contents of *T. testudinum* (leaf C:N explained 100% of the variance, N = 8, with dense mats when $C:N < 22.5$) and sediments (% N explained 91% of the variance, N = 10, with dense mats when $\%N > 11.3$; see also Table 2 and Fig. 3). Plants with lower C:N had more sulfur ($r = -0.691$, p 0.027, N = 10; Fig. 3). No significant correlations between C:N of the leaf tissues and any other element were detected (Table 3). Sediments with higher N contents contained more silt ($r = 0.982$, $p < 0.001$; N = 10). None of the correlations between metals contents in seagrass leaves and the growth form of *H. stipulacea* were significant.

**Table 2**
Sediment characteristics at the study sites together with the growth form of *Halophila stipulacea.* N nitrogen, Org. C Organic Carbon. N 5 (Average ± 95% confidence intervals).

| Sediment characteristic | Sparse | Dense |
|---|---|---|
| % N | 0.06 ± 0.03 | 0.16 ± 0.06 |
| % org. C | 0.58 ± 0.28 | 1.22 ± 0.72 |
| C:N | 10.7 ± 1.7 | 9.3 ± 1.4 |
| D50 (μm) | 222 ± 94 | 135 ± 88 |
| Coarse (% sand) | 19.1 ± 11.7 | 17.3 ± 9.4 |
| Medium (% sand) | 29.2 ± 9.6 | 12.3 ± 7.4 |
| Fine (% sand) | 26.1 ± 11.0 | 16.4 ± 14.3 |
| Silt (% sand ) | 14.7 ± 5.5 | 37.8 ± 14.4 |

## 4. Discussion

The non-native *H. stipulacea* into the Caribbean displayed either sparse or dense growth forms under different environmental conditions. The sparse growth form did not appear to overgrow the local seagrasses *T. testudinum*, *Syringodium filiforme* or *Halodule wrightii*, but rather grew at the edge of the meadow or beneath these seagrasses within the meadow, enhancing the structural diversity by forming extra understory layer. This fast-growing seagrass species may also be an extra food-source for the recovering populations of green turtles (Becking et al., 2015). Thus, although various works have described the non-native *H. stipulacea* as invasive in the Caribbean (DeBrot et al., 2011; Willette and Ambrose, 2009, 2012; Willette et al., 2014), its “invasiveness” (as defined by Verbrugge et al. submitted for publication) likely depends on the environment.

Dense mats were only reported at high environmental nutrient concentrations (as indicated by increased N content in the sediments and *T. testudinum* leaves). We could not establish whether the higher nutrient conditions at the sites were due to natural causes or anthropogenic eutrophication. Heavy metals, which can considered as an indicator of contamination, did not show any significant correlation with the growth form of *H. stipulacea*. Increased silt content in the more eutrophic environments may be a result of nutrient input, but may also be a consequence of the dense growth of *H. stipulacea*. Areas colonized by the introduced seagrass *Z. japonica* in Oregon, northeastern Pacific, showed a decrease in sediment grain size and an increase in richness and densities of resident fauna in comparison with neighboring areas without vegetation (Posey, 1988). The sediments with dense *H. stipulacea* were

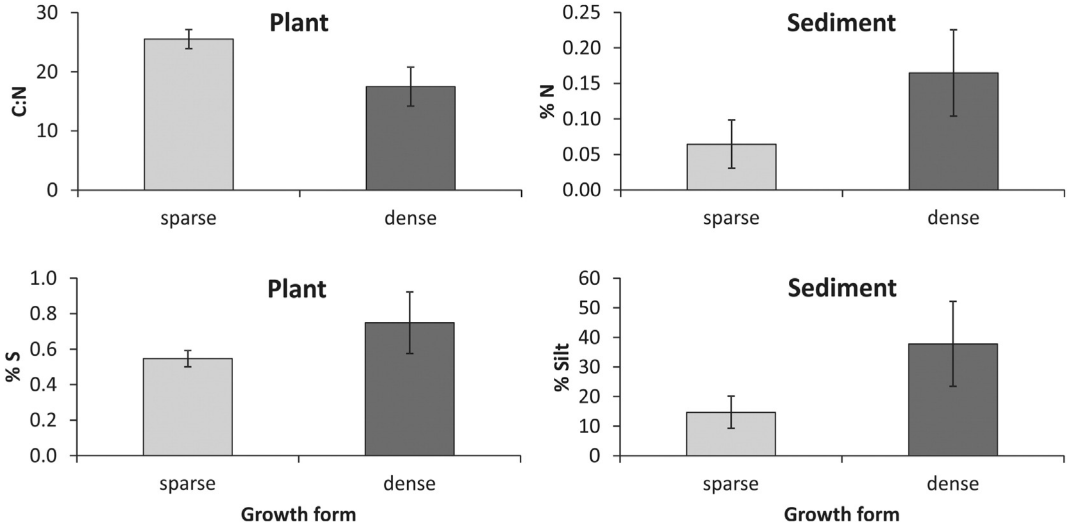

**Fig. 3.** Significantly different plant and sediment characteristics in meadows with sparse or dense growth forms of *Halophila stipulacea* at selected Caribbean islands. C carbon, N nitrogen, S sulfur. Bars represent 95% confidence intervals.

black (Fig. 2C) and smelled of $H_2S$. Seagrasses growing in sulfidic sediments have increased concentration of sulfur compounds in their tissues (Hasler-Sheetal and Holmer, 2015), and *T. testudinum* had higher sulfur content in the leaves when *H. stipulacea* was dense (Fig. 3). This suggest that oxygen was depleted in these sediments; but with present information we can't establish whether this oxygen depletion was the cause or consequence of the dense mats of *H. stipulacea*.

Various authors found indications that dense mats of *H. stipulacea* displaced the native species *S. filiforme* in the Caribbean (Willette and Ambrose, 2009, 2012; Willette et al., 2014). If the more shallow-rooted *H. stipulacea* mats were to replace deeper-rooted species, ecosystem services of the seagrass meadow would be compromised. The shallow-rooted dense *H. stipulacea* mats likely deliver less oxygen into the sediments than the more deeply rooted native seagrass species (Fig. 2D). Deterioration of deeper rooting seagrass species increases the vulnerability of the sea floor to storms and erosion (Cruz-Palacios and van Tussenbroek, 2005). This results in decreased coastal protection (Christianen et al., 2013) as well as decreased carbon sequestration (Marbà et al., 2015).

**Table 3**
Nitrogen (N), Phosphorus (P), Carbon (C) and sulfur (S) in *Thalassia testudinum* leaf tissue content together with concentrations of trace metals. At the study sites together with the growth form of *Halophila stipulacea. Concentrations of the metals in* $\mu g\ g^{-1}$ N 4 (Average ± 95% confidence intervals).

| Plant characteristic | Sparse | Dense |
|---|---|---|
| *Carbon, nitrogen, phosphorus* | | |
| %N | 1.51 ± 0.26 | 2.29 ± 0.30 |
| %P | 0.11 ± 0.01 | 0.13 ± 0.01 |
| %C | 33.2 ± 5.2 | 34.1 ± 2.9 |
| %S | 0.55 ± 0.05 | 0.75 ± 0.17 |
| C:N | 25.5 ± 1.6 | 17.5 ± 3.3 |
| C:P | 834 ± 206 | 704 ± 127 |
| *Metals* | | |
| Cr | 3.3 ± 1.5 | 2.8 ± 2.3 |
| Fe | 108 ± 54 | 96 ± 23 |
| Co | 0.62 ± 0.55 | 0.62 ± 0.49 |
| Ni | 4.3 ± 1.0 | 4.3 ± 0.6 |
| Cu | 5.8 ± 0.9 | 9.4 ± 14.9 |
| Zn | 27.4 ± 25.7 | 35.4 ± 51.7 |
| As | 1.3 ± 0.3 | 1.3 ± 0.1 |
| Cd | 0.2 ± 0.2 | 0.2 ± 0.2 |
| Pb | 0.7 ± 0.4 | 0.6 ± 0.4 |

For seaweeds it is common knowledge that undisturbed marine communities are often initially resistant to most introduced seaweeds (Williams and Smith, 2007; Inderjit et al., 2006). However, non-native seaweeds can become invasive in areas subject to human disturbance (Scheibling and Gagnon, 2006) or increased nutrient input (Ceccherelli and Cinelli, 1997; Steen and Scrosati, 2004; Lapointe and Bedford, 2010). We found that the non-native seagrass *H. stipulacea* in the Caribbean unlikely poses a threat to local seagrasses when sparse. But when nutrients were high, this seagrass formed extremely dense mats with potential invasive properties, which warrants for further monitoring of this species.

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