Exotic *Halophila stipulacea* is an introduced carbon sink for the Eastern Mediterranean Sea

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Carbon and nitrogen storage in exotic *Halophila stipulacea* were compared to that in native *Posidonia oceanica* and *Cymodocea nodosa* meadows and adjacent unvegetated sediments of the Eastern Mediterranean Sea and to that in native *H. stipulacea* of the Red Sea at sites with different biogeochemical conditions and level of human pressure. Exotic *H. stipulacea* possessed considerable storing capacity, with 2-fold higher Corg stock (0.71 ± 0.05 kg m⁻² in the top 20 cm of sediment) and burial (14.78 gC m⁻² y⁻¹) than unvegetated areas and *C. nodosa* meadows and, surprisingly, comparable to *P. oceanica*. N (0.07 ± 0.01 kg m⁻²) and Corg (14.06 ± 8.02 kg m⁻²) stocks were similar between *H. stipulacea* and *C. nodosa* or unvegetated sediments, but different to *P. oceanica*. Corg and N stocks were higher in exotic than native *H. stipulacea* populations. Based on isotopic mixing model, organic material trapped in *H. stipulacea* sediments was mostly allochthonous (seagrass detritus 17% vs seston 67%). Corg stock was similar between monospecific and invaded *C. nodosa* meadows by *H. stipulacea*. Higher stocks were measured in the higher human pressure site. *H. stipulacea* introduction may contribute in the increase of carbon sequestration in the Eastern Mediterranean.

Biological invasion ranks among the most important components of global change, undermining the structure and function of native ecosystems1. The Mediterranean Sea receives the highest number of introduced species globally2 due to the opening of the Suez Canal, high maritime traffic and aquaculture, with 821 marine species being established in the region by year 20163. The seagrass *Halophila stipulacea* (Forsskål) Ascherson 1867, native to the Indian Ocean and the Red Sea4, was first recorded in the Eastern Mediterranean Sea (Rhodes island, Greece) in 1894, following the opening of the Suez Canal in 1869, and since then it has been expanding northward and westward in the basin5 until Tunisia6, usually colonizing unvegetated sediments void of native seagrasses or macroalgae7, with a preliminary estimated mean expansion rate of 12 km y⁻¹. Although the ecological effect of exotic macrophytes is largely assessed8, the impact of *H. stipulacea* on the native ecosystems of the Mediterranean is not yet documented.

*H. stipulacea* is currently considered invasive9, although there is no evidence of competition with the endemic (*Posidonia oceanica* (L.) Delile) and native (*Cymodocea nodosa* (Ucria) Ascherson) seagrasses5, possibly due to its small shoot size compared to that of larger native species. Currently, *H. stipulacea* populations occur only in the warmer southern-eastern part of the Mediterranean basin9, confined by the isotherm of 15 °C, which has been considered the limit of geographical distribution of the species to the West Mediterranean10. However, the occurrence of *H. stipulacea* in the Mediterranean is expanding11,12, possibly following the increase in temperature of the region, where summer sea surface temperature (SST) has raised by 1.15 °C during the last three decades13. Concurrently, *P. oceanica* shows mass mortality due to climatic and anthropogenic stressors14, whereas *C. nodosa* increases its expansion to occupy the available space derived from *P. oceanica* regression15, suggesting a shift in the dominant seagrass of the basin.

Carbon storage in underlying seagrass sediments is a key process in seagrass meadows. Due to intense seagrass metabolism that results in excess organic matter production16 part of which is accumulated in the underlying sediment, it is currently recognized as an important carbon sink globally17,18. However, studies related to the carbon storage in *H. stipulacea* sediments have been rather limited18. The aim of this study is to measure organic carbon and nitrogen storage in exotic *H. stipulacea* meadows and to compare them to those in *P. oceanica* and *C. nodosa* meadows and adjacent unvegetated sediments of the eastern Mediterranean Sea, in two different biogeochemical conditions and level of human pressure.

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The capacity of seagrass ecosystems to store carbon depends on a variety of traits, such as structural complexity, primary productivity, below-ground biomass, capability to trap allochthonous matter and biogeochemical conditions of their sediments. *H. stipulacea* differs in most of the aforementioned traits from the two native seagrass species of the Mediterranean, *P. oceanica* and *C. nodosa*, forming meadows of higher density but of lower below-ground biomass and production than the latter. The simple architecture and low above- and below-ground biomass of *H. stipulacea* imply that the relative sedimentary stock of *H. stipulacea* meadows may be smaller than that of *P. oceanica* meadows, which possess a notable capacity to sequester carbon. Yet, *H. stipulacea* stock may be higher than the usually smaller corresponding stock of unvegetated sediments, due to high shoot density of the species and biomass allocation closer to the interface between water and sediment, which have been related to the high capacity of the genus *Halophila* to trap organic matter.

Although there is a substantial focus in quantifying the capacity of several seagrass species to accumulate organic carbon and the key factors affecting storage, little is known about the amount of inorganic carbon stored in underlying sediments of different seagrass species and the factors determining their storage. Accumulation of inorganic carbon in seagrass sediments derives from sedimentation of carbonate particles, calcification by associated epiphytes and fauna and active precipitation by certain seagrass species, as recently reported for *Thalassia testudinum*. But the magnitude of inorganic carbon deposits depends on the balance between carbonate production and dissolution of carbonates in the canopy or sediment and environmental conditions. Therefore, it is expected to differ across seagrass species and geographic regions, depending on the leaf size, leaf life span, shoot density and production and on the seawater temperature and salinity which regulate the saturation of calcium carbonate (Ω) and hence its precipitation. Likewise, quantification of nitrogen stocks in seagrass ecosystems is puzzling, as nitrogen dynamics in seagrass ecosystems involves a series of biogeochemical processes between the plant, associated flora and fauna, water and sediment (uptake, nitrification, denitrification, fixation) and seagrass communities usually oscillate between being a source or sink for nitrogen.

To our knowledge, the performance of introduced *H. stipulacea* in binding carbon and nitrogen has not been compared yet to that of native seagrass ecosystems. This is important particularly in the context of climate change, where biological invasion in combination with warming and local pressures around the coasts are changing the biogeography of the Mediterranean Sea, which predisposes for relative changes in the magnitude of carbon and nitrogen stored in the aforementioned species across the basin. Here we examine the effect of invasion of exotic seagrass on carbon and nitrogen storage and burial in the Eastern Mediterranean Sea. We do so by comparing carbon and nitrogen storage and burial in exotic *H. stipulacea* meadows to that in endemic *P. oceanica* and native *C. nodosa* seagrass systems and adjacent unvegetated sediments at three sites in Crete island (Greece) characterized by diverse biogeochemical conditions and level of human pressure. We also compare the storage in introduced *H. stipulacea* meadows in the Mediterranean to that at two sites inside the native biogeographic range of the species (Red Sea). Lastly, we examine the isotopic composition of δ13C of seagrass tissues and sediments to identify the contribution of seagrass detritus to the sedimentary organic carbon pool in the Mediterranean and Red Sea sites.

### Results

The structural (shoot density, biomass) and physiological (plant nutrient content) features differed between species and the effect was consistent among sites (Tables 1; S3). *H. stipulacea* formed denser meadows than native species at all sites. Shoot biomass showed the opposite pattern among species, with *H. stipulacea* having the lowest values and being followed by *C. nodosa*, while biomass of both was lower than that of *P. oceanica*. *H. stipulacea* had consistently higher carbon content (45.51–49.53% DW; Tables 2 and S4) than *P. oceanica* (29.36–39.12% DW) or *C. nodosa* (30.16–44.36% DW), but similar nitrogen content (0.56–1.33% DW) across the study sites. *H. stipulacea* was less 13C-depleted than *P. oceanica* and more than *C. nodosa*. Seagrasses leaves were enriched in nutrients at Chania, where also shoot biomass was decreased (Tables 1 and 2).

### Table 1. Mean (±STDEV) of shoot density (shoots m⁻²) and biomass (g DW m⁻²) at each site and species. Capital and small letters indicate significant differences between sites and species, respectively (Tukey’s post hoc test, P < 0.05. The corresponding ANOVA results are given at Table S3).

| Site | Species   | Mean Density (shoots m⁻²) | Mean Leaf biomass (g DW m⁻²) | Mean Rhizome biomass (g DW m⁻²) | Mean Root biomass (g DW m⁻²) |
|------|-----------|---------------------------|-----------------------------|-------------------------------|----------------------------|
| Chania | *H. stipulacea* | 11956 ± 1476Aa | 8.62 ± 2.57Aa | 11.42 ± 1.86Aa | 7.78 ± 2.19Aa |
|       | *P. oceanica*    | 247 ± 57Ab           | 171.57 ± 42.22Ab           | 115.32 ± 35.71Ab           | 67.43 ± 33.93Ab |
|       | *C. nodosa*      | 115 ± 29Ab            | 5.11 ± 0.27Aa             | 11.72 ± 2.54Aa             | 7.63 ± 0.76Aa    |
| Souda  | *H. stipulacea*  | 3132 ± 1013Ba         | 4 ± 0.31Ba                | 4.07 ± 1.42Ba              | 2.66 ± 0.67Aa    |
|       | *P. oceanica*    | 485 ± 180Bb           | 325.68 ± 34.95Bb          | 306.33 ± 107.71Bb          | 86.54 ± 39.72Bb  |
|       | *C. nodosa*      | 491 ± 77Bb            | 13.61 ± 1.42Ba            | 41.57 ± 1.94Bb             | 10.68 ± 1.12Bb   |
| Sitia  | *H. stipulacea*  | 13148 ± 2112Aa        | 6.33 ± 1.56Ba             | 5.75 ± 1.33Bb              | 3.29 ± 0.95Bb    |
|       | *P. oceanica*    | 522 ± 96Ab            | 398.97 ± 49.75Bb          | 446.34 ± 80.61Bb           | 175.16 ± 8.52Bb  |
|       | *C. nodosa*      | 159 ± 43Ab            | 3.48 ± 0.17Ba             | 14.34 ± 3.25Bb             | 11.43 ± 1.06Bb   |
| Site     | Species                          | C leaves (% DW)     | C rhizomes (% DW) | C roots (% DW) | N leaves (% DW) | N rhizomes (% DW) | N roots (% DW) | δ^{13}C leaves (%) |
|----------|----------------------------------|---------------------|-------------------|----------------|----------------|------------------|----------------|------------------|
| Chania   | *H. stipulacea*                  | 48.37 ± 4.56       | 49.09 ± 1.47     | 45.19 ± 1.8a   | 1.28 ± 0.17    | 0.46 ± 0.01     | 0.52 ± 0.04     | −9.3 ± 0.6       |
|          | *P. oceanica*                    | 39.35 ± 0.45       | 39.25 ± 0.77     | 38.74 ± 0.83b  | 0.88 ± 0.09A   | 0.62 ± 0.1A      | 0.43 ± 0.04      | −14.9 ± 0.9     |
|          | *C. nodosa*                      | 36.89 ± 0.53       | 35.54 ± 0.04b    | 30.9 ± 1.32b   | 1.41 ± 0.02    | 0.5 ± 0.02A      | 0.41 ± 0.05      | −6.5 ± 0.3      |
| Souda    | *H. stipulacea*                  | 46.78 ± 2.5       | 46.59 ± 1.13     | 43.15 ± 2.54a  | 0.68 ± 0.41A   | 1.54 ± 1.38      | 0.97 ± 0.48      | −7.8 ± 1.2      |
|          | *P. oceanica*                    | 40.82 ± 1.42       | 34.45 ± 12.83b   | 34.85 ± 14.06b | 1.2 ± 0.38A   | 1.27 ± 0.29B      | 1.21 ± 0.11B     | −13.2 ± 0.4     |
|          | *C. nodosa*                      | 40.31 ± 0.74       | 50.46 ± 8.19b    | 42.31 ± 7.48b  | 0.88 ± 0.5A    | 1.32 ± 0.72B      | 1.8 ± 1.2B       | −6.4 ± 0.1      |
| Sitia    | *H. stipulacea*                  | 49.65 ± 2.05       | 51.7 ± 1.24a     | 47.25 ± 1.27a  | 0.72 ± 0.55B   | 0.85 ± 0.65A      | 0.79 ± 0.54A     | −8.4 ± 0.4      |
|          | *P. oceanica*                    | 33.74 ± 3.89       | 28.97 ± 8.12b    | 25.36 ± 10.45b | 0.59 ± 0.11B   | 0.81 ± 0.12A      | 0.62 ± 0.16A     | −15.8 ± 1.5     |
|          | *C. nodosa*                      | 29.61 ± 2.14        | 34.21 ± 5.68b    | 26.66 ± 8.58b  | 0.52 ± 0.3B    | 0.66 ± 0.43A      | 0.51 ± 0.22A     | −8.2 ± 0.4      |

Table 2. Mean (±STDEV) elemental (%) and isotopic (‰) composition of carbon and nitrogen in seagrass shoots at each site and species. Capital and small letters indicate significant differences between sites and species, respectively (Tukey’s post hoc test, P < 0.05. The corresponding ANOVA results are given at Table S4).

|                              | Df | Mean Square | F-ratio | P-value |
|------------------------------|----|-------------|---------|---------|
| **Two-way ANOVA (Site × Habitat) for Mediterranean Sea region** |    |             |         |         |
| C\text{org} stock            | Site | 2 | 0.022       | 9.57   | 0.001***|
|                              | Habitat | 3 | 0.032       | 13.74  | <0.001***|
|                              | Site × Habitat | 6 | 0.012       | 5.09   | 0.002** |
|                              | Residuals | 24 | 0.003      |         |         |
| C\text{nhog} stock           | Site | 2 | 0.86        | 226.83 | <0.001***|
|                              | Habitat | 3 | 0.03        | 9.20   | <0.001***|
|                              | Site × Habitat | 6 | 0.07        | 18.38  | <0.001***|
|                              | Residuals | 24 | 0.004      |         |         |
| N stock                      | Site | 2 | 0.003       | 7.82   | 0.002** |
|                              | Habitat | 3 | 0.003       | 8.31   | 0.001***|
|                              | Site × Habitat | 6 | 0.003       | 8.28   | <0.001***|
|                              | Residuals | 24 | 0.004      |         |         |
| **One-way ANOVA (Site) for Red Sea region** |    |             |         |         |
| C\text{org} stock            | Site | 1 | 0.02        | 0.08   | 0.798   |
|                              | Residuals | 4 | 0.24       |         |         |
| N stock                      | Site | 1 | 0.003       | 0.78   | 0.428   |
|                              | Residuals | 4 | 0.004     |         |         |
| **Two-way ANOVA (Biogeographic region × Site) for *H. stipulacea* habitat** |    |             |         |         |
| C\text{org} stock            | Biogeographic region | 1 | 43.90      | 113.71 | <0.001***|
|                              | Site | 3 | 0.52        | 1.35   | 0.313   |
|                              | Residuals | 10 | 0.39     |         |         |
| N stock                      | Biogeographic region | 1 | 0.30       | 118.41 | <0.001***|
|                              | Site | 3 | 0.02        | 7.92   | 0.005** |
|                              | Residuals | 10 | 0.003   |         |         |

Table 3. ANOVA results of C\text{org}, C\text{nhog} and N stocks between biogeographic regions, sites and habitats studied.

Granulometry analysis revealed that sediments were mainly sandy with low contribution of silt/clay (3–8%; Table S1), with the exception of South Beach (Red Sea) where 45% was gravel.

The vertical distribution of sediment variables in core profiles was not consistent (Figs S1–S4). Mean carbon and nitrogen content in the first 20 cm of sediment differed among sites, depending on habitat (Tables S5 and S6). *H. stipulacea* sediments were enriched in C\text{org} (mean content across sites 0.32 ± 0.19% DW) and N (0.03 ± 0.01% DW) compared to the unvegetated ones (C\text{org} = 0.18 ± 0.05% DW, N = 0.02 ± 0.002% DW). Again, *H. stipulacea* sediment had higher C\text{org} content than *C. nodosa* (0.18 ± 0.15% DW) although similar N (0.03 ± 0.01% DW),
but lower than *P. oceanica* (C$_{org}$ = 0.49 ± 0.48% DW, N = 0.09 ± 0.11% DW). C$_{inorg}$ content was at the same range between *H. stipulacea* (5.66 ± 2.55% DW) and unvegetated sediments (5.45 ± 2.59% DW) and slightly lower than *P. oceanica* (6.57 ± 5.25% DW) and *C. nodosa* (6.47 ± 33.1% DW) sediments (Table S5). Sediments were enriched in C$_{org}$, C$_{inorg}$ and N at Chania (Table S5). Mean C$_{org}$ and N content at Mediterranean *H. stipulacea* sediments were approximately 2-fold higher compared to Red Sea sites, where they averaged 0.15 ± 0.03 and 0.02 ± 0.01% DW, respectively (Table S5), without showing significant difference between sites of Eilat (Table S6). The inter-correlation of C$_{org}$ and N sediment contents was positive in all species (*H. stipulacea* R$^2$ = 0.85, P < 0.05, *P. oceanica* R$^2$ = 0.87, P < 0.05, and *C. nodosa* R$^2$ = 0.85, P < 0.05).

Stocks of C$_{org}$, C$_{inorg}$ and N in the top 20 cm of sediment differed among habitats, but the effect depended on site (Fig. 1, Table 3). Mean C$_{org}$ stock in *H. stipulacea* across sites (0.71 ± 0.05 kg C$_{org}$ m$^{-2}$ and 35 Mg C$_{org}$ ha$^{-1}$ in top 20 cm and top meter of sediment, respectively) was 2-fold higher than that in *C. nodosa* (0.41 ± 0.08 kg C$_{org}$ m$^{-2}$ and 21 Mg C$_{org}$ ha$^{-1}$ in top 20 cm and 1 m, respectively) and unvegetated habitats (0.42 ± 0.07 kg C$_{org}$ m$^{-2}$ in top 20 cm, 21 Mg C$_{org}$ ha$^{-1}$ in top meter) and similar (i.e. not significantly different) to *P. oceanica* (0.95 ± 0.68 kg C$_{org}$ m$^{-2}$ in top 20 cm, 51 Mg C$_{org}$ ha$^{-1}$ in top meter). Chania supporting higher stocks of C$_{org}$ and N (Fig. 1).

*H. stipulacea* had 2-fold higher N stock (mean across sites 0.07 ± 0.01 kg N m$^{-2}$ in top 20 cm, 3 Mg N ha$^{-1}$ in top meter) than that at unvegetated habitats (mean 0.04 ± 0.01 kg N m$^{-2}$ in top 20 cm, 2 Mg N ha$^{-1}$ in top meter), similar stock to *C. nodosa* meadows (0.06 ± 0.01 kg N m$^{-2}$ in top 20 cm, 3 Mg N ha$^{-1}$ in top meter) and
higher stock from *P. oceanica* in case of Souda and Sitia, although it was lower than the *P. oceanica* mean across sites (0.16 ± 0.18 kg N m\(^{-2}\) in top 20 cm, 8 Mg N ha\(^{-1}\) in top meter).

*H. stipulacea* supported similar (i.e. not significantly different) C\(_{\text{org}}\) stock (14 ± 8 kg C\(_{\text{org}}\) m\(^{-2}\) in top 20 cm, 709 Mg C\(_{\text{org}}\) ha\(^{-1}\) in top meter) to *C. nodosa* (15 ± 9 kg C\(_{\text{org}}\) m\(^{-2}\) in top 20 cm, 771 Mg C\(_{\text{org}}\) ha\(^{-1}\) in top meter) and unvegetated habitats (14 ± 9 kg C\(_{\text{org}}\) m\(^{-2}\) in top 20 cm, 691 Mg C\(_{\text{org}}\) ha\(^{-1}\) in top meter), but significantly higher stock to that of *P. oceanica* (12 ± 9 kg C\(_{\text{org}}\) m\(^{-2}\) in top 20 cm, 590 Mg C\(_{\text{org}}\) ha\(^{-1}\) in top meter).

There was no significant difference among C\(_{\text{org}}\) stock in monospecific *C. nodosa* (Chania, Souda) and *C. nodosa* meadows invaded by *H. stipulacea* (Sittia) (ANOVA, F = 4.7, P = NS).

Stocks (top 20 cm of sediment) of *H. stipulacea* were similar within Red Sea sites (Table 3), with 0.36 ± 0.03 kg C\(_{\text{org}}\) m\(^{-2}\) and 0.04 ± 0.008 kg N m\(^{-2}\) at North Beach and 0.35 ± 0.06 kg C\(_{\text{org}}\) m\(^{-2}\) and 0.04 ± 0.006 kg N m\(^{-2}\) at South Beach.

Stocks of C\(_{\text{org}}\) and N in *H. stipulacea* sediments differed between biogeographic regions (Table 3; Tukey’s post hoc test Chania ≠ Souda) by almost 2-fold. They ranged between 0.55 and 0.79 kg C\(_{\text{org}}\) m\(^{-2}\) with a mean of 0.71 kg C\(_{\text{org}}\) m\(^{-2}\) and 0.05 and 0.08 kg N m\(^{-2}\) with a mean of 0.07 kg N m\(^{-2}\) at Mediterranean Sea sites and between 0.28 and 0.39 kg C\(_{\text{org}}\) m\(^{-2}\) with a mean of 0.36 kg C\(_{\text{org}}\) m\(^{-2}\) and 0.03 and 0.05 kg N m\(^{-2}\) with a mean of 0.04 kg N m\(^{-2}\) at Red Sea sites (Fig. 2).

According to the mixing model, most of the sedimentary C\(_{\text{org}}\) in the meadows examined derived from allochthonous sources (i.e. SPOM and/or *Cystoseira* sp.), with their contribution ranging from 42% at Chania to 86% at Sitia (mean = 66%) in the Mediterranean meadows and from 70% at North Beach to 84% in South Beach (mean = 77%) in the Red Sea meadows (Fig. 3). *C. prolifera* present only at Chania sites contributed with 29% in mean. Among seagrass species, *H. stipulacea* showed the lowest contribution to sedimentary C\(_{\text{org}}\), ranging from 13% at Chania and Sitia to 26% at Souda (mean of 17%), compared to *C. nodosa*, that contributed 9%, 39% and 14% at Chania, Souda and Sitia, respectively (mean of 21%) and *P. oceanica*, that showed the highest contribution, contributing 30%, 41% and 25% at Chania, Souda and Sitia sedimentary stocks, respectively (mean 32%). *H. stipulacea* detritus contributed by 30% and 16% at North and South Beach, respectively, with a mean of 23% at Red Sea sites. Posterior distributions of possible solutions for all end-members used in each Bayesian mixing model (i.e. for the three habitats of Chania and Sittia in presence of *H. stipulacea*) are reported in Fig. 56.

Based on the \(^{210}\text{Pb}\) activities (Fig. 4), sediment accumulation rate at Chania was 0.39 cm y\(^{-1}\) in *H. stipulacea* and 0.3 cm y\(^{-1}\) in *C. nodosa* meadows and 0.2 cm y\(^{-1}\) in unvegetated sediment. The derived age of the 20 cm sediment depth corresponded to approximately 51 y in *H. stipulacea*, 100 y in unvegetated and 67 y in *C. nodosa* meadow. The burial rate of C\(_{\text{org}}\), C\(_{\text{inorg}}\) and N was 2–3 times higher at *H. stipulacea* meadow (14.78 gC\(_{\text{org}}\) m\(^{-2}\) y\(^{-1}\), 237 gC\(_{\text{inorg}}\) m\(^{-2}\) y\(^{-1}\) and 1.09 gN m\(^{-2}\) y\(^{-1}\)) compared to those at the unvegetated sediment (4.91 gC\(_{\text{org}}\) m\(^{-2}\) y\(^{-1}\), 134 gC\(_{\text{inorg}}\) m\(^{-2}\) y\(^{-1}\) and 0.39 gN m\(^{-2}\) y\(^{-1}\)). The C\(_{\text{org}}\) burial rate of *H. stipulacea* was again 2-fold higher but C\(_{\text{inorg}}\) and N burial rates were similar to that of *C. nodosa* (6.46 gC\(_{\text{org}}\) m\(^{-2}\) y\(^{-1}\), 281 gC\(_{\text{inorg}}\) m\(^{-2}\) y\(^{-1}\) and 0.99 gN m\(^{-2}\) y\(^{-1}\)).

**Discussion**

The exotic *H. stipulacea* meadows studied here supported notable sedimentary C\(_{\text{org}}\) stocks compared to native seagrass, as well as to adjacent unvegetated sediments. Higher stocks in seagrass compared to bare sediments has been reported elsewhere, with 3 to 11-fold higher C\(_{\text{org}}\) stocks of *P. sinuosa* meadows in Australia\(^{27}\) and 2 to 4-fold higher stocks of *Thalassia testudinum* and *Halodule wrightii* meadows in Gulf of Mexico\(^{28}\) than the corresponding bare sediments. Mean C\(_{\text{org}}\) content in *H. stipulacea* sediments was similar to values reported from Greece before (0.37 ± 0.3% DW\(^{29}\)). *C. nodosa* sediments were particularly poor in sedimentary C\(_{\text{org}}\) with a mean content similar to estimates from Cadiz Bay (Spain) (0.15% DW\(^{30}\) Supplement Material) and Greece (0.25 ± 0.3% DW\(^{29}\)), which resulted in lower sedimentary stock. There are no available estimates of *C. nodosa* stocks around Mediterranean, but further studies are necessary given the current progression of species distribution\(^{2}\) and also the potential shift in its distribution following change in thermal conditions of the basin\(^{29}\). Mean C\(_{\text{org}}\) in *P. oceanica* sediments were on the same range of that from Greek waters before (0.4 ± 0.19% DW\(^{34}\) and 0.35 ± 0.3% DW\(^{29}\)). C\(_{\text{org}}\) was
comparable to data from Italy (3.09 ± 2.2% DW, eastern Sicily; S. Vizzini, unpubl. data), but still ranged lower than the mean from Spain (3.91% DW, N = 21755), which possibly represents the most complete compilation of relative data, suggesting large variability in C_{org} content in *P. oceanica* sediments, which is reflected in the high variability of stocks calculated for the species across Mediterranean. The mean (across sites) sedimentary stock of *P. oceanica* here was lower than the mean (167 ± 65 Mg C_{org} ha^{-1} in the top meter) from previous measurements obtained in Western Mediterranean meadows (Balearic Islands) and even less than that in *P. oceanica* reefs (372 ± 65 Mg C_{org} ha^{-1} from the same region, where the plant grows vertically for many meters down into the

**Figure 3.** Percentage contribution of end-members (grouped when their δ^{13}C was not significantly different, see Materials and methods section) to the first 5 cm of sediment organic carbon of each habitat and site investigated. Each box contains 50% of the data, the thick horizontal line indicates the median; lower and upper whiskers of the boxes represent respectively the lowest and the highest values of the total range of the distribution. Black diamonds show the mean value, black triangles show the mode, where available.
yet, the surprisingly comparable sedimentary $C_{org}$ stock of $H. stipulacea$ to that of $P. oceanica$ meadows suggest considerable capacity of the former species to accumulate carbon in relation to its native counterpart. In accordance with our results, the stocks of meadows formed by the congeneric species of those of our study, $H. ovalis$ and $P. australis$ in Australia, did not differ despite the big difference in their shoot size, and $H. ovalis$ stock was the second highest among 10 seagrass species studied there\(^1\). This result was attributed to the high capacity of $Halophila$ to trap organic matter, but also to its preference to naturally inhabit depositional environments. This is consistent with the results found in $H. stipulacea$ meadows examined in this study, where sediments were mainly composed of seston and less of seagrass detritus (67\% vs 17\% contribution to organic matter pool, respectively), suggesting that high carbon sequestration capacity of the species depends more on the deposition and trapping of allochthonous material\(^1\) and less on its below-ground biomass and productivity, which are at the lower end range provided for seagrass species\(^1\).

The high capacity of $H. stipulacea$ to trap allochthonous matter possibly relates to its high density and small size. $Halophila$ sp. reach high densities and $H. stipulacea$ in particular forms far denser meadows (12,795–25,345 shoots m\(^{-2}\); 10,500 ± 2,700 shoots m\(^{-2}\); 476–9,900 shoots m\(^{-2}\); 1,506–6,092 shoots m\(^{-2}\), this study) than $P. oceanica$ or $C. nodosa$ (e.g. 244–700 shoots m\(^{-2}\) and 544–2,331 shoots m\(^{-2}\), respectively\(^2\), this study). In addition, the smaller leaves of $H. stipulacea$ than other seagrass species (Leaf Area Index = 0.82, 0.95 and 6.39 m\(^{2}\) leaves m\(^{-2}\) for $H. stipulacea$, $C. nodosa$ and $P. oceanica$, respectively\(^2\)) and the relatively simple architecture of $H. stipulacea$ with leaf and rhizome biomass allocation closer to interface between sediment and water may result in significant sediment stabilization effects, as shown for the congeneric species $H. decipiens$\(^3\). An experimental study showed that Caulerpa sp. beds, an alga of small size similar to $H. stipulacea$, had equal or even higher capacity to trap particles than $P. oceanica$ or $C. nodosa$ beds, as small size and high density of Caulerpa sp. shoots induced a ‘skimming flow’ over the beds and increased transport of particles to the canopy\(^4\). In our case, the 2 to 3-fold higher burial rate of $H. stipulacea$ meadow compared to that of unvegetated and $C. nodosa$ habitats, a rate that actually falls in the reported range for the $P. oceanica$ meadows of Balearic Islands, Western Mediterranean (9 and 52 g$C_{org}$ m\(^{-2}\) y\(^{-1}\)) vs $H. stipulacea$ beds, an alga of small size similar to $H. stipulacea$, had equal or even higher capacity to trap particles than $P. oceanica$ or $C. nodosa$ beds, as small size and high density of Caulerpa sp. shoots induced a ‘skimming flow’ over the beds and increased transport of particles to the canopy\(^4\).

The capacity of $H. stipulacea$, but also of the native species, to store N was substantial. To our knowledge, despite the increase in $C_{org}$ stock data, there are only a couple of estimates of N stock in any seagrass species. A mean estimate of 12.4 ± 1.1 Mg N ha\(^{-1}\) has been provided, encompassing values from Thalassia testudinum meadows across Florida Bay and Amphibolis antarctica and Halodule uninervis meadows from Shark Bay\(^4\). A modeled seagrass recovery following a large restoration effort in coastal bays of Virginia yielded 170 ton N yr\(^{-1}\) via denitrification\(^5\), suggesting a significant removal of N from the ecosystem by the holobiont. Despite the parallel accumulation of organic carbon and nitrogen in sediments, the trend of $C_{org}$ and $N$ stocks between habitats differed, suggesting species specific differences. Content of N in $H. stipulacea$ and $C. nodosa$ sediments were at a similar range with values from the Eastern Mediterranean before (0.04 ± 0.03% DW and 0.02 ± 0.03% DW, respectively\(^6\)), but sediment N in $P. oceanica$ was higher than previously reported (0.03 ± 0.01% DW\(^7\) and 0.04 ± 0.01% DW\(^8\)), resulting in significant higher N stock in the latter species. Very recently, the quantification of N stock of several Z. marina meadows across Denmark showed that this stock is highly variable in space (24–440 gN m\(^{-2}\)), depending on sediment characteristics (i.e. grain size) and nutrient availability\(^9\). Complex N dynamics in seagrass ecosystems, namely N mineralization and fixation\(^9\), along with high variability of N in tissues of different seagrass species\(^10\), which are both affected by species specific differences and environmental conditions\(^9\), could result in highly variable N content in underlying sediments\(^11\) and thus diverse stocks across species and regions, but further studies are needed to elucidate this.
The higher amount of $C_{\text{org}}$ but similar $C_{\text{inorg}}$ stored between *H. stipulacea* and unvegetated habitats suggest that the accumulated $C_{\text{inorg}}$ does not derive solely from calcification inside the meadow. Environmental factors such as temperature and salinity favor carbonate precipitation by other benthic organisms inhabiting or visiting the meadow86. $C_{\text{inorg}}$ stock of *H. stipulacea* meadows fell very close to the global mean for seagrasses (654 ± 24 Mg $C_{\text{inorg}}$ ha$^{-1}$), but it was far higher than the mean value calculated for *Halophila* genus so far (304 Mg $C_{\text{inorg}}$ ha$^{-1}$), expanding the range of $C_{\text{inorg}}$ stock for the genus. Despite the smaller leaf size and life span24,25 and lower load of calcareous epiphytes in *H. stipulacea* meadows, the $C_{\text{inorg}}$ stock was higher compared to that in *P. oceanica*, suggesting that shoot density more than shoot size affected the storing capacity here. In fact, accumulation of $C_{\text{inorg}}$ was considerable also in *C. nodosa* compared to *P. oceanica*, both supporting similar stocks given for the corresponding genus (738 Mg $C_{\text{inorg}}$ ha$^{-1}$ and 563 Mg $C_{\text{inorg}}$ ha$^{-1}$, respectively88). A global review of $C_{\text{inorg}}$ stocks in seagrass ecosystems did not find a clear effect of genera size on the amount of $C_{\text{inorg}}$ stored, with some small genera supporting large stocks and *Posidonia* in particular supporting intermediate stocks89.

The higher $C_{\text{org}}$ and N stocks of *H. stipulacea* meadows across sites, but also of both native species, were measured at Chania, where the meadows receive the highest human pressure among the studied sites, as shown by the highest Pressure Index measured at this site44 and the mesotrophic conditions. An increase in $C_{\text{org}}$ burial was also measured at *P. oceanica* meadows of Mallorca (Spain) since the onset of anthropogenic pressure and particularly at sheltered areas with high human activity44, which was related to the increase in contribution of seston to the organic pool of seagrass sediments as a result of general eutrophication associated with the intensification of coastal anthropic activities85. Here we did not measure any increase in the SPOM contribution at Chania, but we did observe rich macroalgal communities (*Caulerpa prolifera*), which contributed, on average, by 29% to the sediment organic pool, and mesotrophic conditions, as shown by the relatively high Chl concentration in the water column, suggesting relatively nutrient enriched conditions at the specific site. This was also consistent with higher sediment $C_{\text{org}}$ and nutrient content measured in seagrass tissue at Chania, which is indicative of increased nutrient availability to seagrass meadows90.

The lack of significant difference among $C_{\text{org}}$ stocks in monospecific *C. nodosa* and invaded meadows by *H. stipulacea* suggests that the invasion of *H. stipulacea* did not affect negatively the carbon stock of the natives. Nevertheless, a progression in its distribution could be expected as a result of warming67, which may trigger replacement of the latter that would result in a substantial reduction in type and amount of ecosystem services29. A higher sediment Corg and nutrient content measured in seagrass tissue at Chania, which is indicative of increased nutrient availability to seagrass meadows65.

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Table 4. Sampling design and site characteristics (depth (m) and grain size (% sand — % silt/clay)). *Data from77.

| Biogeographic region | Site                          | Habitat | Time   | Depth |
|----------------------|-------------------------------|---------|--------|-------|
| Mediterranean Sea    | Chania (35°3′55″N 24° 4′48″E) | H. stipulacea | June 2013 | 16 m  |
|                      |                               | Unvegetated |       | 16 m  |
|                      |                               | P. oceanica |       | 21 m  |
|                      |                               | C. nodosa   |       | 16 m  |
|                      | Souda (35°26′17″N 24° 8′54″E) | H. stipulacea | June 2013 | 5 m   |
|                      |                               | Unvegetated |       | 5 m   |
|                      |                               | P. oceanica |       | 5 m   |
|                      |                               | C. nodosa   |       | 5 m   |
|                      | Sitia (35°12′26″N 26° 0′18″E)  | H. stipulacea | Sept 2013 | 10 m  |
|                      |                               | Unvegetated |       | 10 m  |
|                      |                               | P. oceanica |       | 10 m  |
|                      |                               | C. nodosa   |       | 10 m  |
| Red Sea              | North Beach (29°32′46″N 34°57′53″E) | H. stipulacea | June 2014 | 9 m   |
|                      | South Beach (29°29′51″N 34°54′45″E) | H. stipulacea | June 2014 | 9 m   |

buried in the region. Furthermore, the weak rhizome structure of the species, particularly as opposed to that of P. oceanica, suggests enhanced probability of sediment erosion and subsequent loss of sedimentary C_organic stock. Lastly, a better understanding of the effect of this exotic species on the native biodiversity, and, importantly, how this effect may change in the context of future warming of the region are a prerequisite, before we can account the full size of H. stipulacea sedimentary stock in the Mediterranean Sea.

Materials

Sampling strategy. The study was conducted at Crete Island, Greece (Mediterranean Sea) and Eilat Bay, Israel (Red Sea) (Table 4). We selected three sites in Crete (Chania, Sitia and Souda), where all seagrass species formed monospecific stands (except Sitia, where H. stipulacea expanded inside some C. nodosa patches), and two sites at Eilat (North Beach and South Beach), where only H. stipulacea was present. We sampled all seagrass species and adjacent unvegetated sediments at each site in Crete and only H. stipulacea in Eilat. Seagrasses were the only macrophytes present at the sites, with the exception of Chania, where Caulerpa prolifera and Cystoseira spp. were found adjacent to the seagrasses. The sites were visited during the warm season, when temperature range was 20–26 °C. The depth range was 5–21 m, well above the lower depth limit of the seagrass (90 m81), and sediments were mainly sandy.

The sites were characterized by different level of human pressure. Chania is under moderate human pressure by cumulative impacts (i.e. sewage discharge, agriculture run-off, industrial/chemical pollution, eutrophication and harbor/marina/ports) with a Pressure Index (which quantifies all the pressures exerted in the water bodies) of 0.7864. Souda is also affected by similar pressures (sewage, agriculture, industry, maritime traffic) but to a lower extent (Pressure Index = 0.5664). Pressure Index is not available for Sitia, but the site should be considered unaffected, situated in a non-urbanized bay with no coastal activity. North Beach is under high human pressure, namely extended coastal infrastructures and highly populated beaches, while South Beach is relatively unaffected. The annual mean chlorophyll a (Chl_a) concentration of the water column (satellite data; Oceancolor web Aqua MODIS L3-SMI 4 km) during the corresponding sampling years (2013 and 2014 for Mediterranean and Red Sea sites, respectively) for Chania, Souda, and Sitia and for North and South Beach was 0.12 μg l⁻¹, 0.15 μg l⁻¹, 0.09 μg l⁻¹, 0.17 μg l⁻¹ and 0.16 μg l⁻¹, respectively, classifying the particular sites as lower mesotrophic (Chl_a = 0.1–0.4 μg l⁻¹), except Sitia which was classified as oligotrophic (Chl_a < 0.1 μg l⁻¹)82.

Shoot number of P. oceanica and C. nodosa was measured in situ by divers at each site from five randomly thrown 40 cm × 40 cm quadrates. Divers also collected P. oceanica and C. nodosa shoots by hand (3 replicates, 10 shoots per replicate), to measure biomass at the laboratory. Shoot density and biomass of H. stipulacea were estimated at the laboratory from shoots collected by divers using an aluminum core (5 replicates, i.d. 15 cm).

Sediment cores (3 replicates, i.d. 4.5 cm D each) were collected from each site down to 20 cm sediment depth, to allow the reconstruction of recent sediment records. Sediment was sliced in various depth intervals (0–1, 1–5, 5–10, 10–15 and 15–20 cm). Sediment compaction, which is inevitable when coring, was measured as the difference between the outer and the inner distance from the top of the core to the sediment surface divided by the core length inserted in the sediment and was very small (3–6%).

Laboratory analysis. The seagrass shoots were transferred to the laboratory where the leaves were gently scraped with a razor blade to remove epiphytes, and seagrass modules (leaves, rhizomes, roots) were dried at 60 °C for 48 h. Dried tissue was acidified (HCl, 2 N) to remove carbonates. Carbon and nitrogen content and δ13C were analyzed in an Elemental Analyzer (Thermo Scientific Flash EA 1112) connected to an Isotope Ratio Mass
Sediment from each slice was dried at 60 °C for 48 h and ground. Grain size analysis was performed using wet sieving to separate the sand fraction (>63 μm), whereas the finer silt and clay fractions (63 to 0.1 μm) were analyzed with a Sedigraph (Micromeritics 5100).

Dried sediment samples were weighed in silver (for organic carbon, Corg analysis) and tin (for total carbon, Ctot and total nitrogen, N analysis) capsules and analyzed as above. Corg and N were analyzed on sediment as it is, while Corg was analyzed on sediment acidified with HCl (18%) added drop by drop to remove carbonates. Inorganic carbon (Cinorg) was estimated as the difference between Ctot and Corg. δ13C was analyzed in acidified (HCl, 2 N) sediment. The analytical precision of the δ13C measurement based on the standard deviation of replicates of the internal standard Vienna Pee Dee Belemnite was 0.1‰.

Calculations. Shoot density (shoots m−2) was estimated as the number of shoots divided by the sampled area for each species.

Biomass (g DW m−2) of each seagrass module was estimated as the product of dry weight per shoot and shoot density.

Dry bulk density (g cm−3) was calculated as the dry weight of sediment divided by the volume of the wet sample.

Stock of organic and inorganic carbon and nitrogen (g cm−3) at the top 20 cm of sediment was estimated as:

\[
\text{Stock} = \Sigma (C_i \times b_i \times d_i),
\]

where \(C_i\) is the concentration of Corg or Corg or N (in % DW divided by 100), \(b_i\) is the dry bulk density (in g cm−3) and \(d_i\) is the sediment depth (in cm) of the sediment slice \(i\). The stocks were converted to kg m−2 and Mg ha−1 to compare with similar studies.

For the calculation of the sediment accumulation rates, the down core total 210Pb activity was determined through the activity of its alpha-emitting granddaughter 210Po, assuming secular equilibrium with 226Ra. For the total dissolution of the dried sediments the analytical method described by83 was applied. The supported 210Pb activity was determined through the activity of its parent 226Ra (assuming they are in equilibrium), which was measured in a High Purity Germanium detector (HPGe) with nominal relative efficiency 50% (ORTEC GEM-FX8530P4). The 210Po activity was calculated from the difference between the total 210Pb activity and that of the background. For the calculation of the rates the Constant Flux Constant Sedimentation model (CFCS)84 was used. The down core activities of 210Po were measured in all the study sites, however, in most cases it was not feasible to calculate any accumulation rate as the 210Pb activities were too low or the sediment cores were bioturbated. The supported 210Pb activity was determined only in the case of Chania (H. stipulacea, C. nodosa and unvegetated), in order to calculate the relative rate. Due to the coarse character of the sediments and the short length of the cores, it was not possible to use 137Cs as an independent tracer.

Data analysis. The Shapiro-Wilk test was used to check if the data were normally distributed. Cochran's test was used in order to check the heterogeneity of variance in seagrass and sediment variables prior to performing Analysis of Variance (ANOVA). Data were log-transformed when necessary. A two-way ANOVA was used to detect possible statistical differences between sites and habitats or biogeographic regions85. In case of significant difference between levels (P < 0.05), a Tukey's post-hoc test was used to show which level differed. Regression analysis was used in order to detect a trend in the distribution of sediment properties with depth. The aforementioned analyses were performed using R version 3.3.3 (R Core Team, 2017).

Mixing models were used to estimate the contribution of potential sources of organic matter to the carbon isotopic composition (δ13C) of sediment, hence to elucidate the origin of Corg present. Mixing models were restricted to the first 5 cm of sediment (hence using data available for 0–1 and 1–5 cm layers); deeper and older layers were excluded because they may have undergone isotopic alterations during post-depositional decomposition of seagrass tissues, which instead are considered null or negligible in surficial layers (e.g.56,57). Models were run separately for each site and habitat. Only end-members present at each site and habitat were included in the models as observed during sample collection. These were: different species of seagrasses, suspended particulate organic matter (SPOM) as a proxy for phytoplankton, and macroalgae (Caulerpa prolifera and Cystoseira spp. present only at Chania habitats). Seagrass δ13C data were analyzed in the present study, with the exception of those of H. stipulacea from the Red Sea sites, which were provided by M.C. Gambi, G. Winters, S. Vizzini (unpubl. data, Table S2). The δ13C signature of SPOM was obtained from the literature, by averaging values of Mediterranean coastal areas87,88 (Table S2). Macroalgae δ13C data, specifically for Caulerpa prolifera and Cystoseira spp. were obtained respectively from S. Vizzini (unpubl. data) and87 (Table S2). When the number of end-members considered was two (one seagrass species and SPOM), a two-source mixing model was adopted based on the following equation by89:

\[
\text{fraction of source } 1 (%) = \frac{\delta^{13}C_{\text{sediment}} - \delta^{13}C_{\text{source 2}}}{\delta^{13}C_{\text{source 1}} - \delta^{13}C_{\text{source 2}}} \times 100.
\]

This was the case of all the three habitats of Souida, two out of three habitats of Sitia (P. oceanica and C. nodosa habitats) and both the Red Sea sites. When the number of end-members considered was higher than two, Bayesian mixing models were used (R package SIAR: Stable Isotopes Analysis in R90). This was the case of the three habitats of Chania and the H. stipulacea habitat of Sitia. Differences in δ13C among end-members were tested through Permutational Analysis of Variance (PERMANOVA) based on the Euclidean distance matrix and when they were non-significant (P > 0.05) end-members were grouped according to91 to reduce sources of uncertainty that could influence mixing model results when using multiple end-members. The only end-members grouped were SPOM and Cystoseira spp. at the three habitats of Chania.
Data Availability
Data supporting this study will be available upon request to EA.

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Author Contributions
E.A. conceived and designed the study. E.A., S.V., C.A. performed the sampling. E.A., S.V., V.S., C.A., H.K. performed the laboratory analysis. E.A., S.V. and C.A. analyzed the data. E.A., S.V., H.K. and E.P. contributed materials and analysis tools and wrote the paper.

Additional Information
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