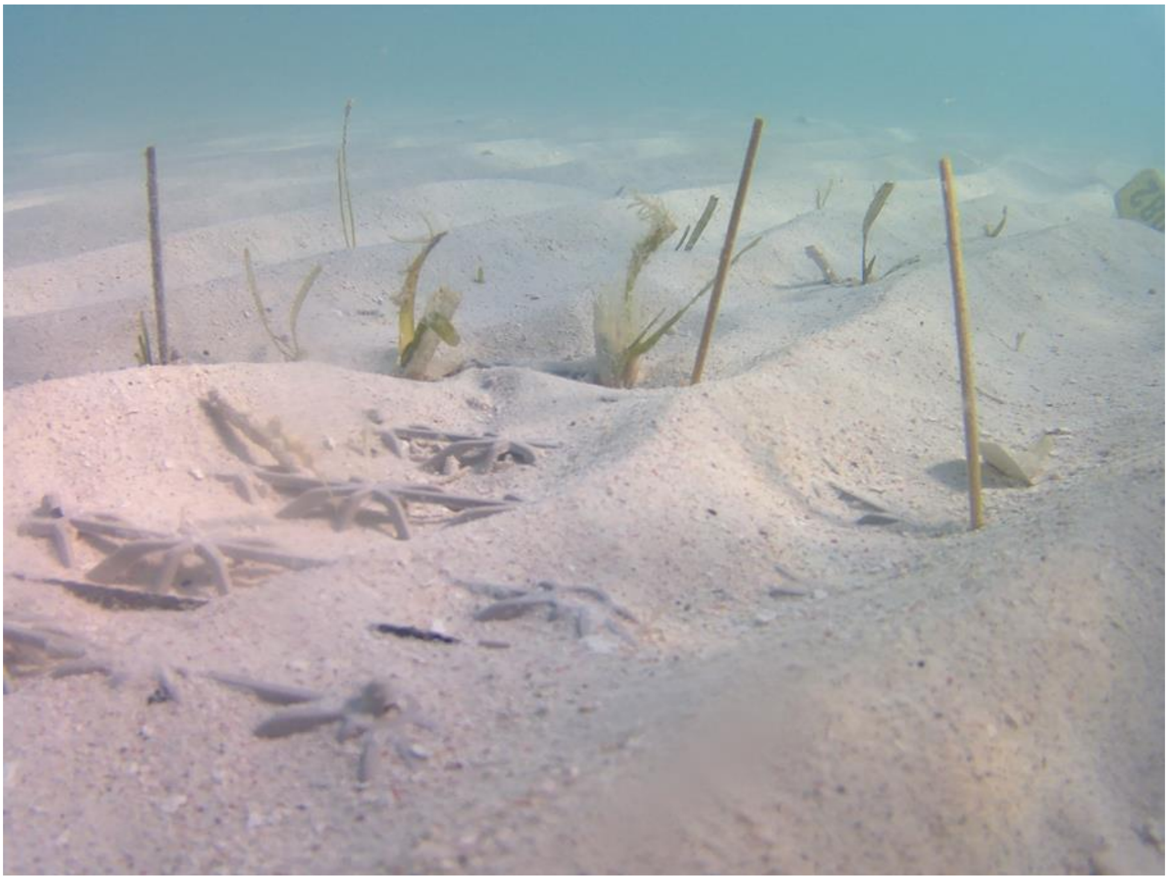
Shifting the balance between native and invasive seagrass through novel restoration methods, in Lac Bay, Bonaire



MSc thesis by Anniek Vos
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Aquatic Ecology and Water Management

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Master thesis Aquatic Ecology and Water Management Group submitted in partial fulfilment of the degree of Master of Science in Aquaculture and Marine Resource Management at Wageningen University, the Netherlands

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Abstract

Seagrass is of great importance worldwide for coastal protection, carbon sequestration and as a nursery and feeding habitat for various species. However, due to climate change, eutrophication, turtle grazing and anthropogenic activities seagrass meadows are declining globally. Seagrass restoration might be a tool to restore the seagrass ecosystem and bring back the linked ecosystem services. In the case the area is ought to be suitable for restoration, different restoration methods can be used. This study will focus on the importance of sediment stabilisation for seagrass restoration of the native seagrass *Thalassia testudinum* and the invasive *Halophila stipulacea*, using biodegradable sheets that mimic the sediment stabilizing function of seagrass meadows. This study is executed in Lac Bay, Bonaire. It is expected that by using these stabilizing sheets, the balance between native and invasive seagrass can be shifted towards native seagrass occurrence. During this research we found that using sediment stabilizing root mats can improve restoration of the native seagrass *T. testudinum*, especially in environments with high wave action and currents. Sediment stability is provided and fragments are held in place by the use of these biodegradable sheets, which prevents fragments from washing away. However, for the long-term these biodegradable sheets are possibly negatively affecting seagrass growth, likely due to interference with rhizome growth. This should, however, be researched into further detail. The invasive seagrass species *H. stipulacea* does not experience advantages in terms of growth when using these root mats. Fragments of *H. stipulacea* are fragile and possibly suffer from different kinds of stress when implementing in between the sheets. It could be stated that by using the sediment stabilizing sheets, the balance between native and invasive species can be shifted towards native seagrass in this research. This will benefit the seagrass ecosystem and its ecosystem services. In general it can be stated that the effect of using these biodegradable sheets differs depending on the seagrass species and various environmental factors such as hydrodynamics. There is also an indication of a difference in efficiency of the use of these sheets between the short-term and long-term growth. Furthermore bioturbation is likely to influence seagrass expansion and the functionality of these biodegradable sheets, therefore further research is advised.

Keywords: *seagrass restoration, BESE products, Thalassia testudinum, Halophila stipulacea, Bonaire, growth, survival, lateral expansion, hydrodynamics, bioturbation*

Preface

During all the phases in this research I received great support from various people and organisations. First of all I would like to thank Fee Smulders and Marjolijn Christianen for giving me this amazing opportunity and having trust in me. I would specifically like to thank my supervisor Fee Smulders for all her support, advice and quick responses on urgent questions while being on Bonaire. Next to that I would like to thank the team of researchers from Wageningen University, Bureau Waardenburg and NIOZ consisting out of Marjolijn Christianen, Fee Smulders, Karin Didderen and Tjisse van der Heide for their support and shared knowledge during the first week on Bonaire. This also accounts for Sabine Engel, who was part of this team as well. Thank you Sabine for all your time, patience and help with various things, from arranging transport to providing knowledge, research materials and a helping hand. Next to that I would like to thank STINAPA for their help with logistics and assistance, as well as the people of Mangrove Maniacs for their offered help. Last but definitely not least, a big thank you to my friend Shamyi Lanjouw who was always there for me and always helped with fieldwork.

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1. Introduction

Seagrasses are flowering marine plants that grow underwater (Orth et al., 2006). Seagrasses are adapted from the land to the nearshore marine environment and spread along worldwide coastlines (Short & Coles, 2001). Almost all flowering seagrasses consist out of above and belowground parts. The belowground part consists out of rhizomes and roots which anchor the seagrass. The aboveground part consists out of shoots which consist out of several leaves (Larkum et al., 2006). Worldwide about 72 different species exist (Short et al., 2011). Despite the limited diversity in seagrasses, seagrass is an important contributor to coastal marine ecosystems. Seagrass is of great importance worldwide for coastal protection and as a nursery and feeding habitat for various species (Christianen et al., 2013; Govers et al., 2014). Seagrass induces sedimentation by their aboveground leaf canopy and reduces erosion since the seagrass meadows attenuate the impact of waves on the seafloor and shore (Borum et al., 2004). Seagrass is not only important for coastal protection, it also serves as a habitat and food supply for many species (Temmink et al., 2020). For example turtles feed on seagrass and some fish species use the seagrass meadows as their nursery habitat (Christianen et al., 2013; Govers et al., 2014; Smulders et al., 2017). Other than that seagrass plays an important role in carbon sequestration. Seagrass namely accounts for 10% to 18% of the total oceanic carbon burial, while only 0.1% of the ocean floor is covered with seagrass (Greiner et al., 2013; Winters et al., 2020). The above mentioned statements make clear that seagrass provides many important ecosystem services.

Due to different causes, such as eutrophication, fishing and recreation, grazing of turtles and climate change, the global cover of seagrass meadows is declining (Cullen-Unsworth et al., 2014; Govers et al., 2014). This has a negative impact on the seagrass ecosystems since seagrass is a source of food and shelter for many species, as well as their other ecosystem services provided above. Humans and nature are thus harmed by the decrease of seagrass (Govers et al., 2014; Temmink et al., 2020).

In areas where seagrass disappeared or is declining, restoration might be a tool to restore the seagrass ecosystem and bring back the linked ecosystem services (Paling et al., 2009). However, before starting a restoration project, the cause of decline of seagrass needs to be clear (Orth et al., 2006). When this is known it can be assessed if seagrass restoration is possibly effective or if first certain stressors need to be reduced (Paling et al., 2009). In the case the area is ought to be suitable for restoration, different restoration methods can be used. Examples are implementation of seeds and transplanting seagrass shoots in a clumped design (Greiner et al., 2013; Temmink et al., 2021). These methods can be expensive and prone to failure since the focus lays on facilitating a single life stage, while bottlenecks are experienced throughout multiple life stages (Temmink et al., 2021). Another constraint of these methods is that for transplanting seagrass many transplants are needed in order to simulate the self-stabilizing function of dense seagrass meadows. This self-stabilizing function means that sediment is stabilized due to dense seagrass cover. When seagrass transplants are not transplanted dense enough they can easily be swept away by wave action or currents (Temmink et al., 2020; van Katwijk et al., 2016).

Therefore, a relatively new restoration method that focusses on overcoming the first sediment stability barrier is introduced. With this method restoration is done by using implementation structures called BESE structures. BESE stands for Biodegradable EcoSystem Engineering Elements. These structures mimic the sediment stabilizing function that seagrass meadows have, which later on enables the seagrass to be self-sustainable. The BESE structures are made out of waste from the potato industry and consist of 100% biodegradable material (*BESE Products*, 2021; Temmink et al., 2020).

This project is part of the mangrove and seagrass restoration project in Lac Bay, on Bonaire, in the Caribbean. In this project the Dutch Ministry and STINAPA cooperate with Bureau Waardenburg and Wageningen University (*BESE Products*, 2021). Lac Bay is a bay with a surface of 7,5 km^2 located at the east side of Bonaire (Govers et al., 2014). This bay has the largest seagrass beds of the Caribbean and is an important forage area for turtles and nursery habitat for fish (Becking et al., 2014). Multiple seagrass species are present in this bay, both native and invasive species. The two most abundant being the native species *Thalassia testudinum* and the invasive seagrass species *Halophila stipulacea* (Smulders et al., 2017). The invasive seagrass *H. stipulacea* was introduced and established in Lac Bay in 2010. Between 2011 and 2017 the abundance of this species has increased in Lac Bay from about 6% to 26% occurrence per m^2. At the same time the native seagrass *T. testudinum* decreased from 51% to 34% occurrence per m^2 (Christianen et al., 2019). The invasive seagrass has multiple disadvantages compared to the native seagrass species. Namely smaller roots that are shallower rooted and thus provides less in coastal protection (Duarte et al., 1997; Malm, 2006; Winters et al., 2020). The seagrass canopy of *H. stipulacea* is shorter than the canopy of the native meadows, this increases predation risk for species which can be associated with a lower species abundance (Becking et al., 2014). Another disadvantage of this invasive seagrass is its lower nutritional value for turtles (Christianen et al., 2019). However, settlement of *H. stipulacea* is not necessary negative. When *H. stipulacea* settles on bare areas, this increases for example sediment stability and fish diversity (Viana et al., 2019). Furthermore, fish graze on *H. stipulacea* which could limit the invasion success of *H. stipulacea* (Smulders et al., 2022). However, if *H. stipulacea* overtakes *T. testudinum* this could have negative consequences for the seagrass ecosystem in Lac Bay (Becking et al., 2014; Smulders et al., 2017; Winters et al., 2020).

Therefore, it is important to investigate if by using BESE restoration structures settlement of the native *T. testudinum* can be favoured over settlement of the invasive *H. stipulacea*. The objective of this research is to determine whether BESE structures are a favorable tool to stimulate the growth of native seagrass over invasive seagrass.

The main research question is: What is the impact of BESE structures on the restoration success of the native seagrass *T. testudinum* and the invasive *H. stipulacea*?
To answer this question the following specific research questions need to be answered:

1. What is the impact of BESE structures on growth of the native *T. testudinum*?
2. What is the impact of BESE structures on growth of the invasive *H. stipulacea*?
3. What is the impact of BESE structures on growth of both native *T. testudinum* and invasive *H. stipulacea* in mixed plots?
4. What is the difference in impact of BESE structures on native and invasive growth between wave exposed locations and locations with little to no waves?

2. Methodology

2.1. Area description

This research experiment was set up in Lac Bay, a shallow bay situated in the southeast of Bonaire. Southern Caribbean, which includes around 200 hectares of seagrass meadows (Christianen et al., 2019; Smulders et al., 2017). The dominant seagrass species in the bay are *T. testudinum* (*figure 1c*)*,* *H. stipulacea* (*figure 1d*) and *S. filiforme* (Christianen et al., 2019). In the northeast of the bay, two small research areas, shown in *figure 1ab*, were set up in bare areas and the research plots were placed within those research areas. On location A more wave movement as well as ripples on the ocean floor were visible than on location B.

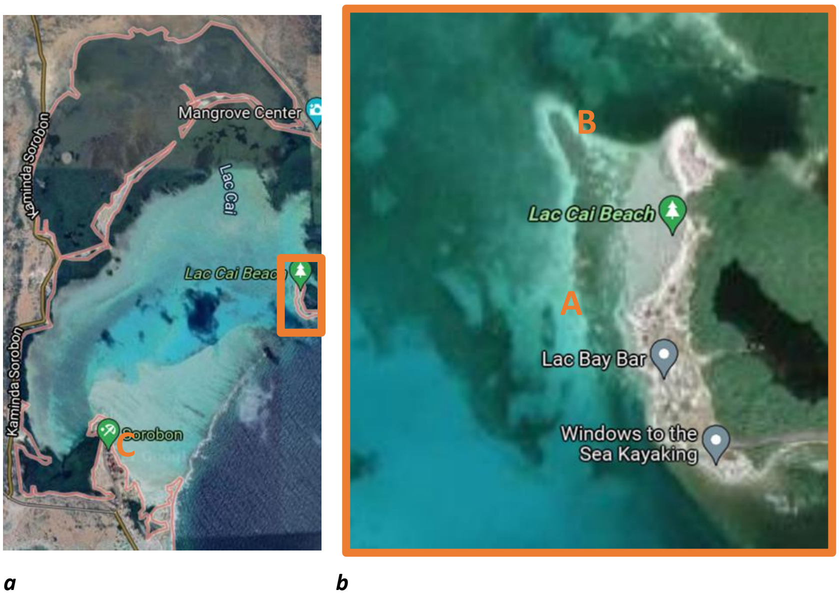

*Figure 1a: Lac Bay, at location C T. testudinum fragments were taken. 1b: The square on 1a zoomed in. At location A more wave action and currents are present in comparison with location B (*Map Lac Bay*, 2022).*

The average measured water temperature at the locations was approximately 28°C when looking at the months December until February (own measurements). The depth between and within the research locations varies. The research location with less wave action and currents (location B in *figure 1b*) is a shallow area with depths varying between plots from about 0.5 meter depth to 1.5 meter depth. The other research location (location A in *figure 1b*) is a deeper area with plots varying in depth from about 1.5 to 2 meters depth (own measurements).

2.2 Experimental set up

Experimental treatments of location A and B are visualized in *figure 2&3*. Of each different treatment, four replicates were made. These different plots were completely randomized per location.

figure 1c: Thalassia testudinum fragments

figure 1d: Halophila stipulacea fragment

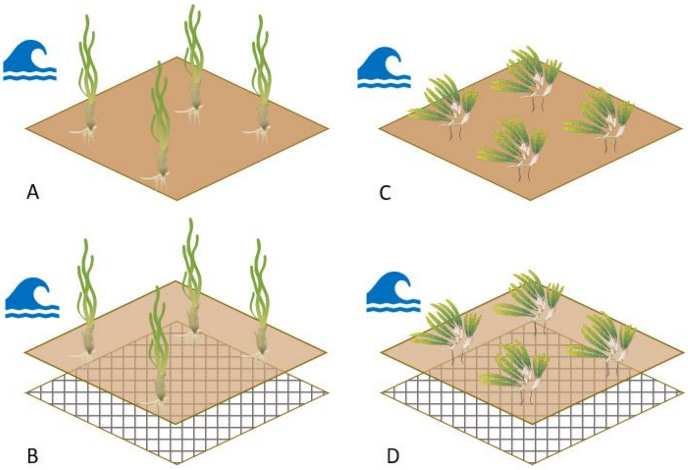

Figure 2: *Different plot combinations present at location A.*
A *plot with native T. testudinum in sediment.* ***B*** *plot with native T. testudinum in BESE structure.* ***C*** *plot with invasive H. stipulacea in sediment.*
D *plot with invasive H. stipulacea in BESE structure.*

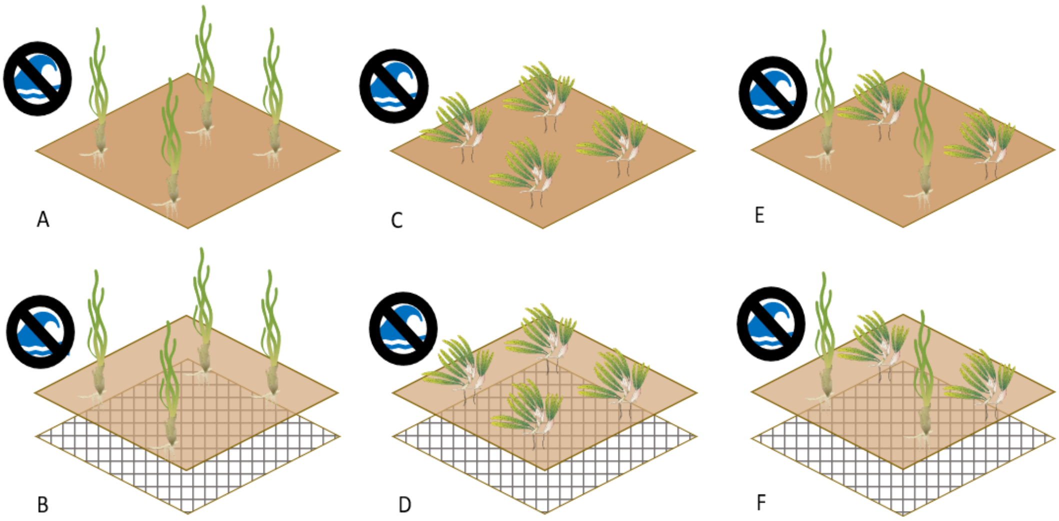

Figure 3: *Different plot combinations present at location B.* ***A*** *plot with native T. testudinum in sediment.* ***B*** *plot with native T. testudinum in BESE structure.* ***C*** *plot with invasive H. stipulacea in sediment.* ***D*** *plot with invasive H. stipulacea in BESE structure.* ***E*** *plot with mixed seagrass species in sediment.* ***F*** *plot with mixed seagrass species in BESE structure.*

First the plots at the less exposed location B were set up. Every plot has the size of a BESE sheet, which have the following measures: 91.5 × 45.5 × 2 cm and create a surface of roughly 0.4 m^2 (*BESE Products*, 2021; Temmink et al., 2020). Multiple sheets can be stacked on top of each other to create the wanted design. In this case two sheets were placed on top of each other with the seagrass transplants in between since this was done and tested before (MacDonnell et al., 2022). Per plot six fragments of seagrass with two or three shoots per fragment were added. Only fragments with an apical meristem were used and implemented with the apical tip pointing outwards. This was done because *T. testudinum* fragments with a rhizome with an apical meristem are initially more productive than *T. testudinum* fragments without an apical meristem (Fonseca et al., 1998; Marbà & Duarte, 1998). Fragments of *T. testudinum* were taken out of Lac Bay at a place called Sorobon which is marked with a C in *figure 1a*. These transplants were taken out there because at that place is easily accessible and the density of apical meristems is high. Fragments of *H. stipulacea* always have multiple apical tips and were taken out of the surroundings of location A (*figure 1b*). For *H. stipulacea* six fragments were placed per plot, with four shoots per fragment. In the mixed plots three fragments of each species were placed with the abovementioned number of shoots per fragment per species.

At location B six different treatments with four replicates each were implemented (*figure 3*). Per plot the rhizome length and number of shoots were measured of every fragment. In plots using BESE structures fragments were clicked between two BESE layers and dug into the sediment to simulate the function of a sediment stabilizer, as dense natural seagrass meadows have (Temmink et al., 2020). Two pins of steel were placed to hold the sheets in place. Control plots were marked with two pins of steel and rhizome length and number of shoots per fragment were measured underwater. Hereafter fragments were dug into the sand a bit. In Annex 1 you can see a map of the underwater plots at location B. The plots were placed all with a minimum distance of two meters between each other in the direction of the main currents and with a minimum distance of one meter between each other perpendicular on the direction of the main currents. This was done to avoid plots influencing each other (Temmink et al., 2020).

Later, new plots were set up in an area with more wave action and currents. This area will be called location A from now on. Plaster sticks (*figure 4*) were used to compare wave action and currents in this location with location B.

figure 4: a plaster stick underwater

At this location different treatments were implemented as can be seen in *figure 2*. Again, four replicates of the different treatments were made. The implementation of these plots was done the same way as described above for location B. In Annex 2 a map is displayed of this research area.

After fragments washed away (see results section) new fragments of *H. stipulacea* were implemented in all plots in both locations, but this time with two wooden pins per fragment that were used to hold the fragment on its place in the sediment (*figure 5*). This method was used to test if a part of the fragments would stay in place and be able to settle and grow by using wooden pins. Next to that it was done to be able to make comparisons between locations and treatments of plots containing *H. stipulacea.*

figure 5: H. stipulacea implemented with two wooden pins to try to hold fragments on its place

2.3 Monitoring

The plots in both research areas were monitored weekly. During this weekly monitoring various factors were measured. These are factors that indicate growth as well as environmental factors that could influence growth. Weekly or biweekly measured were number of shoots per fragment per plot, number of leaves per shoot, sediment mobility by measuring sediment pins, fish presence and rhizome growth. Next to this other factors were measured less frequently, such as leaf productivity, bioturbation activity, sediment bulk density, temperature, salinity, oxygen availability and nutrient composition in porewater. Due to the high amount of collected data, choices were made in which data would be most valuable to analyse. The process of collecting and analysing this data is described in this section 2.3.3.

2.3.1 Growth

In order to assess the outcome of the research questions, growth was determined by looking at the difference in **number of seagrass shoots (shoot growth)** and the **lateral expansion of seagrass (rhizome growth)** (Temmink et al., 2020)**.** Together with this also **fragment survival** was compared between locations and treatments as well as **leaf productivity** (Short & Coles, 2001).

Fragment survival

Differences in fragment survival between locations and treatments can show the effects of different environmental conditions and restoration methods on seagrass survival (Ferretto et al., 2021). Fragment survival was compared between locations, treatments and mono versus mixed plots together with treatments for both seagrass species. At the time of implementation of the plots, per plot six fragments were implemented. The number of fragments still present per plot were counted again during the final monitoring session. This was expressed in a survival percentage of fragments per plot and compared between locations and treatment per seagrass species. This fragment survival percentage was also compared between mono versus mixed plots together with treatments for both seagrass species.

Lateral expansion

Lateral expansion of the rhizome is another indicator of growth (Short & Coles, 2001; Temmink et al., 2020). Lateral expansion was compared between locations, treatments and mono versus mixed plots together with treatments for both seagrass species. As mentioned before, the length of the rhizome of each fragment was measured when the fragments were implemented. At the end of the experiment two fragments per species per plot (if still present) were taken out and rhizome length was measured again. These fragments were randomly chosen out of the ones that were still there and from which the rhizome was still intact. The difference in length divided by the number of days between the begin and end measurement, this results in the lateral growth per day. The mean lateral growth per day per plot was compared between locations and treatment per seagrass species. Next to that, lateral growth was also compared between mono versus mixed plots together with treatments for both seagrass species.

Shoot growth

Expansion of number of shoots was used as an indicator of seagrass growth (Short & Coles, 2001). During weekly monitoring the number of shoots were counted per fragment. In order to do this fragments and shoots were numbered per plot as can be seen in a schematic overview of a BESE plot in *figure 6*. The change in number of shoots per plot after settlement was compared to the number of shoots in the last monitoring round. The comparison was made between locations and treatments per seagrass species as well as between mono versus mixed plots together with treatments for both seagrass species. The monitoring date defined as the first monitoring round after settlement differs per seagrass species and per location. In Annex 3 an overview is given of these and other important dates. Settlement dates are chosen based on first signs of new shoot development and stabilisation of fragment presence.

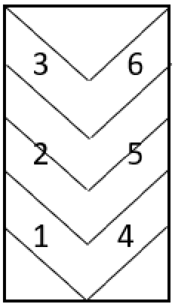

figure 6: schematic overview of fragment numbering in a BESE plot

Leaf productivity T. testudinum

Leaf productivity was measured twice, once around December to test the method and the final measurements were done at the end of January. This was done to test possible differences in leaf productivity between the different locations and treatments. Leaf productivity was measured at *T. testudinum* shoots by using the hole-punch method (Short & Coles, 2001). A detailed description of the hole-punch method can be found in Annex 4. Leaf productivity was measured of plots at location A and B and of natural shoots in both Lac Cai and Sorobon.

With the obtained data the percentage of new leaf growth of the total leaf length was tested between both research locations and treatments. Difference in the percentage of new leaf growth per day was tested as well between treatments and mono versus mixed *T. testudinum* plots. Next to this, the mean new growth per day was also given for all four sites as well as leaf surface.

Since leaf productivity was only measured for *T. testudinum* shoots, for *H. stipulacea* shoots leaf surface was compared between treatments and research locations to give an indication of difference in leaf growth.

2.3.2 Environmental factors

Next to factors that define growth, multiple environmental factors were measured. This gives useful information about different conditions between research locations, treatments and/or seagrass species, with potential impacts on seagrass growth.

Wave action

Wave action is important when selecting sites for seagrass restoration (Borum et al., 2004; Hotaling-Hagan et al., 2017; Paling et al., 2009). Therefore, two research locations were chosen to conduct this experiment and test differences in seagrass survival and growth between those two locations and also between treatments (BESE plots versus control plots). These locations needed to be compared in terms of wave action which was done by using the plaster stick method. Four plaster sticks were randomly spread at each research location. 24 hours after the placement they were recovered again, dried and weighed. The difference in dry weight before and after deployment indicates the amount of wave action and current strength. This deploying and retrieving was done three times in total. The differences in weight from the plaster part were tested to see if there is a significant difference in wave action between both research locations.

Bulk density sediment

Bulk density of sediment is correlated to sediment dynamics and influences seagrass growth and settlement (Suykerbuyk et al., 2016). To measure the difference in bulk density of the sediment at both research locations, four sediment samples at each location were taken at a random spot within the research location. These sediment samples were taken by using a 20 mL syringe that was cut off at the top. This syringe was pushed vertically into the sediment until it was filled up until 20 mL with sediment. From this weight the bulk density was calculated the data was analysed to see if there was a difference in bulk density between locations.

Water temperature

Since water temperature can influence seagrass growth as well (Borum et al., 2004), water temperature was measured continuously at both research locations for about two weeks. This was done by using a HOBO logger which was available for two weeks. The logger was placed in the middle of both research locations and measured both water temperature (in degrees Celsius) and light intensity (in lux) every half an hour. This data was converted to an excel sheet, averaged per day and data from the 18th of January until the 30th of January was used to compare the temperature between both locations. This was ought to be long enough to have a sufficient amount of data. However, sediment might have landed on the HOBO logger on the ocean floor which could influence results. Therefore, the temperature and light intensity measurements of the first five days only were tested as well.

Light intensity

Light is an important factor for seagrass to grow. Without light photosynthesis could not take place and seagrasses could not grow (Borum et al., 2004; Carr et al., 2016; Dennison, 1987). Light intensity was measured continuously at both research locations as well for about two weeks by using the HOBO logger. Again, the data was converted to an excel sheet, averaged per day and data from the 18th of January until the 30th of January was used to compare light intensity between both locations. Measurements of the first five days only were tested as well.

Bioturbation

Bioturbation activity was measured because of its potential impact on seagrass settlement and expansion (Suykerbuyk et al., 2016; Valentine et al., 1994). Small holes as shown on the photograph in *figure 7* were counted once for every plot at both locations. This was done by throwing a quadrant of 23 by 23 cm on each plot and then counting all the holes inside. This data was analysed to see if there was a significant difference of bioturbator holes between both research locations and treatments.

Next to that also four core samples were randomly taken at each research location. This was done by using a pvc tube with a diameter of 15 cm and a length of 30 cm with a net on one side. This tube was pushed into the ocean floor about 20 cm deep and then as fast as possible turned upside down. The sediment present in this tube was put into a mesh bag and taken to shore where it was sorted out to see if there was any fauna present. This was done to be able to see if there is a possible influence of infauna on the growth of seagrass.

figure 7: bioturbator holes in a research plot

Porewater

Porewater was measured to be able to compare presence of nutrients, oxygen and salinity, which influences seagrass growth, between locations and treatments (Borum et al., 2004). Porewater was subtracted by using syringes of 50 mL on which rhizons were attached. Rhizons are small tubes that filter out bacteria from water. These syringes with rhizons were placed in each plot combination at each location multiple times. About 30 to 40 mL of water was taken per plot.

Dissolved oxygen was measured in milligrams per litre directly at the shore (Handy Polaris, Oxyguard). After this the porewater samples were analysed on salinity, phosphorus and nitrogen-nitrite content. **Salinity** was measured in grams of salt per kilo of seawater (ppt) (EC salinity meter). **Phosphorus and nitrogen-nitrite concentrations** were measured by using a nutrient kit (Hanna marine pocket photometer nitrite and phosphorus). The device can measure concentrations between 0 and 200 ppb.

The data from all the different factors measured from the porewater was gathered again in an excel sheet and differences in dissolved oxygen, salinity, phosphorus and nitrogen-nitrite concentrations between locations, treatments and seagrass species were analysed. The outcome of this is useful information for growth comparisons between the different locations and treatments. If these values differ per location this could influence the growth as well and needs to be taken into account.

Sediment mobility

About once every two weeks sediment-burial pins, which measure sediment stability, were measured. This is done to be able to compare differences in sediment mobility between locations and treatments since this could influence seagrass survival and could be influenced by different treatments (Paling et al., 2009; Temmink et al., 2020). This is a pin of stainless steel that was placed in the sediment with a ring around it. This ring lays on the sediment at the beginning, the distance of the top of this pin to the sediment (and thus ring) was measured. Every two weeks the distance from the top of the pin to the sediment and from the top to the ring was measured again to get to know more about the sediment dynamics at this location. In total ten pins were placed, one on every plot combination at location B and one on every plot combination in location A. This data was summarized again in excel where the difference between begin measurement and end measurement of the distance between the top of the pin and the sediment was calculated per plot. The difference between the biggest and smallest distance between the sediment and top of the pin was also calculated per plot. Thirdly, the sediment mobility was measured by measuring the average distance between the ring and the sediment per plot. These differences were analysed and compared per location and treatment.

Surface water samples

Water samples were taken a few times as well at both research locations. These were taken in a bottle which is pushed underwater and then turned upside down so water flows in. Dissolved oxygen, temperature and salinity were measured. This was again done to compare possible differences between locations and link this to seagrass growth (Borum et al., 2004). **Dissolved oxygen** was measured in saturation percentage as well as in dissolved milligrams per litre. This was measured by using the same measuring device as used to measure dissolved oxygen in the section 'porewater' as described above. **Temperature** was measured as well with the device that also measures dissolved oxygen. Temperature was measured in degrees Celsius. **Salinity** was measured by using the same salinity meter as described in the section 'porewater'. This meter measures salinity in the amount of grams of salt per kilo of seawater which is the same as in parts per thousand (ppt).

2.3.3. Data analysis

A software program called Rstudio version 1.3.1093 was used for statistical analysis of data. Depending on the type of data and tested variables the executed test differed. The factors considered in this field experiment were location, seagrass species and substrate. The Shapiro-Wilk test was used to examine normality of the data together with looking at Q-Q plots. The Levene's test was used to examine equal variance(s) of the data as well as residual-vs-fitted plots. Depending on these outcomes a test was assigned. When multiple variables were compared, depending on the amount of variables, a two-, or three-way ANOVA was used. In the case data did not have a normal distribution, a log transformation was made. If after a log transformation data still did not have a normal distribution or variances were unequal, a ranked ANOVA was performed. When only one variable was tested and data had no normal distribution (even after a log transformation) an ANOVA was performed. When data had a normal distribution, equal variances and an equal sample size, a t-test was performed. In the case data did not have equal variances, and/or no equal sample size, a Welch t-test was performed. In table 1 and 2 an overview of performed tests is given.

3. Results

First the results of different factors describing growth are presented and explained. After this the results of different environmental factors are described. Together these results answer the question how the implementation of BESE structures influences the balance between the native seagrass *T. testudinum* and the invasive seagrass *H. stipulacea*.

3.1 Growth

Growth results are described below per seagrass species by using results of fragment survival, lateral expansion, shoot growth and leaf productivity. Fragments of *H. stipulacea* washed away and died in both locations. Eventually at location A all the *H. stipulacea* fragments were gone when monitoring the 3rd of January. In location B there was only one fragment of *H. stipulacea* left on the 10th of January. Therefore, on the 12th of January new fragments were implemented using a different method as is described in the method section.

3.1.1 Fragment survival

T. testudinum

A ranked ANOVA is performed which shows there was no significant difference in fragment survival between locations and/or treatments. However, when we look at the p-value describing a possible interaction between treatments and mixed and mono plots and *figure 8* we can see there is a possibility that an interaction took place (f(1)=4.3, p=0.06). In the boxplot displayed in *figure 8* it is shown that in location B fragment survival was slightly higher in control plots while at location A fragment survival was much lower in control plots than in BESE plots.

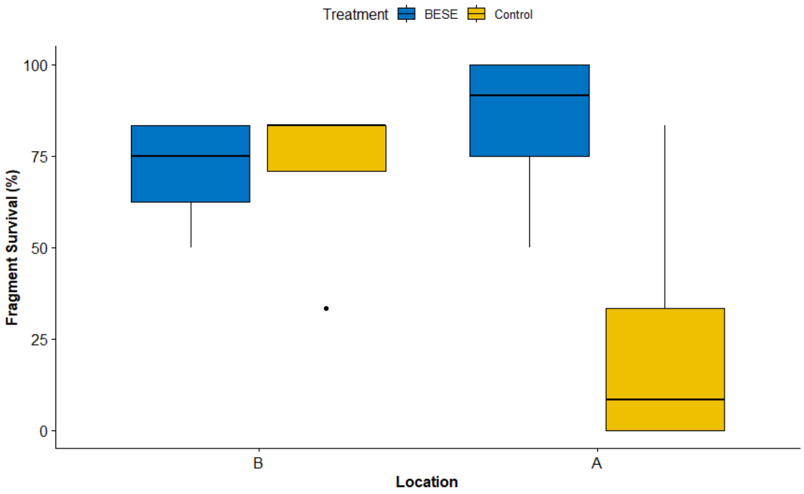

figure 8: Fragment survival between research locations and treatments

H. stipulacea

A two-way ANOVA was performed to test differences in fragment survival of *H. stipulacea* between locations and treatments. A significant difference was found in fragment survival between treatments (f(1)=16.7, p=0.00) which means fragment survival was higher in control plots. This can be seen in Annex 5A.

Mix vs. mono T. testudinum

In this case a ranked ANOVA was used to test differences in fragment survival of *T. testudinum* between treatments and mix/mono plots. This test shows a significant interaction between treatments (BESE and control) and mix/mono plots (f(1)=7.0, p=0.02). In the boxplot in Annex 5B it is shown that in BESE plots the fragment survival of mixed plots was higher while in control plots fragment survival of mono plots was higher.

Mix vs. mono H. stipulacea

A significant difference in fragment survival of *H. stipulacea* between treatments was found (two-way ANOVA, f(1)=9.4, p=0.01). In this case fragments of both mono and mixed plots had a higher survival in control plots, this is shown in a figure in Annex 5C.

3.1.2 Lateral expansion

T. testudinum

A two-way ANOVA was performed to compare lateral expansion between locations and treatments. This resulted in no significant difference in growth of *T. testudinum* between locations and/or treatments (treatments: f(1)=3.7, p=0.09). At location A only one control plot still contained *T. testudinum* fragments that could be measured to determinate lateral expansion. This lack of data gives difficulties to compare lateral expansion between treatments and locations. Therefore, lateral expansion was compared between treatments at location B only. No significant difference in lateral expansion between treatments was found (Welch t-test, t(4.7)=-2.5, p=0.06). As indicated by the p-value and the graph in *figure 9*, there was a possible difference in lateral expansion between the treatments. Although it was not statistically proven, there is an indication that in control plots with *T. testudinum* faster lateral growth occurred.

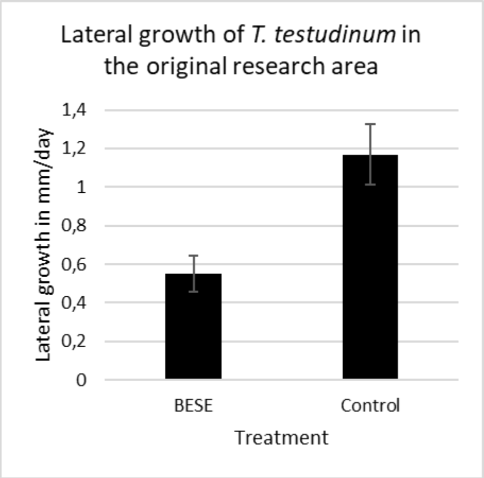

figure 9: Lateral growth between treatments at location B

H. stipulacea

A two-way ANOVA was performed to compare lateral expansion of *H. stipulacea* between locations and treatments. This ANOVA showed a significant difference in lateral expansion between treatments (f(1)=8.0, p=0.02). It is shown that there was a faster lateral growth of *H. stipulacea* fragments in the control plots. This can be seen in the boxplot in Annex 5D.

Mix vs. mono T. testudinum

A ranked two-way ANOVA was performed to test differences in lateral growth of *T. testudinum* between mixed and mono plots and treatments. This resulted in a significant difference in lateral growth between treatments (f(1)=12.0, p=0.01). Once again it was shown that rhizomes grow faster in control plots. This is also visible in Annex 5E.

Mix vs. mono H. stipulacea

In this case a two-way ANOVA was performed to test differences in lateral growth of *H. stipulacea* between mixed and mono plots and treatments. This resulted in a significant difference in lateral growth between both treatments (f(1)=18.6, p=0.01) and mono and mixed plots (f(1)=10.3, p=0.03). Lateral growth was higher in control plots and in mixed plots. This is shown in a boxplot in Annex 5F.

3.1.3 Shoot growth

T. testudinum

Shoot growth of *T. testudinum* was tested between research locations and treatments (BESE and control plots. A two-way ANOVA showed a significant interaction between treatment and location (f(1)=6.2, p=0.03). The differences in shoot growth between both treatments (BESE and control) differed per location. When looking at *figure 10* shoot growth seems equal between BESE and control plots at location A, while at location B more growth in control plots is shown. Next to that there was a significant difference in added shoots between research locations (f(1)=8.0, p=0.02). Looking at the boxplot in *figure 10* more shoots were added (or less shoots disappeared) at location B. Next to this, there is an indication that there might be a difference in shoot growth between treatments, however this is not significant (f(1)=4.6, p=0.053).

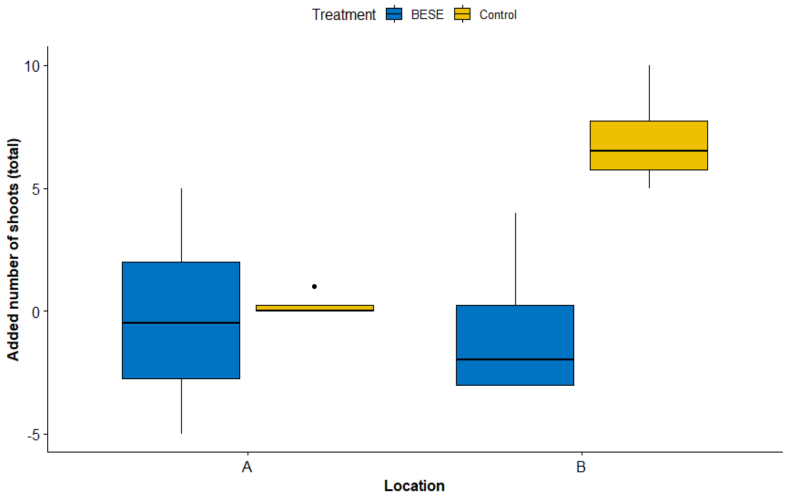

figure 10: Shoot difference of T. testudinum between location and treatment

Due to the significant interaction and seeming difference in shoot growth between treatments at location B, both locations were tested separately. For both locations a t-test was done. At location A there was no significant difference in shoot growth between treatments, however at location B there was (t(5.2)=-3.9, p=0.01) with a higher shoot growth in control plots.

H. stipulacea

Shoot growth of *H. stipulacea* was tested between research locations and treatments (BESE and control plots). No significant difference in shoots growth between treatment and location were found, however there was an indication for a possible difference between treatments (two-way ANOVA, f(1)=4.3, p=0.06), with a higher shoot growth in control plots (*figure 11*).

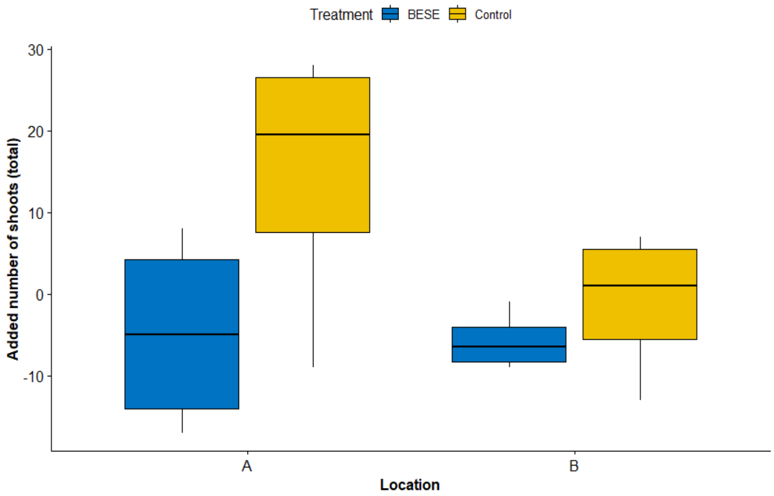

figure 11: Shoot difference of H. stipulacea between location and treatment

Mix vs. mono T. testudinum

Shoot growth of *T. testudinum* was tested between mono and mixed plots and treatments (BESE and control plots). A significant interaction between mono and mixed plots and treatments is found (two-way ANOVA, f(1)=9.3, p=0.01). There was also a significant difference between both treatments (f(1)=10.6, p=0.01) and mono and mix plots (f(1)=9.3, p=0.01). In *figure 12* the interaction can be seen, in control plots there was a higher shoot growth in mono *T. testudinum* plots. However, in BESE plots shoot growth was higher in mixed plots. From this figure it can also be seen that there was a higher shoot growth in control plots.

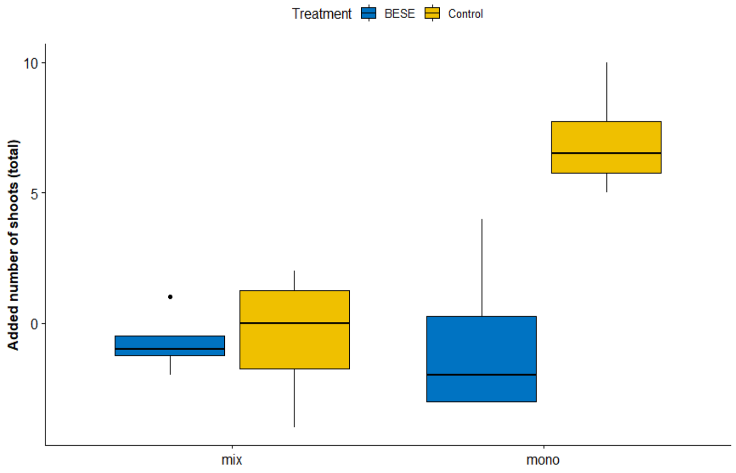

figure 12: Shoot difference of T. testudinum between mono and mix plots and treatment

Mix vs. mono H. stipulacea

Shoot growth of *H. stipulacea* was tested between mono and mixed plots and treatments (BESE and control plots). The performed two-way ANOVA shows there was no significant difference between mono and mixed plots and treatments.

3.1.4 Leaf productivity *T. testudinum*

When new growth per day was tested between locations and treatments, no significant differences were found. However, there was a significant difference in growth between both locations when the percentage of new leaf growth over total leaf length was tested between locations and treatments (two-way ANOVA, f(1)=7.9, p=0.02). At location A *T. testudinum* grew faster which is shown in a boxplot in Annex 5G.

Mix vs. mono

A two-way ANOVA was performed to test the difference in percentage of new leaf growth between mono and mixed plots and treatments. This resulted in no significant differences in growth between mono and mix plots and treatments.

New growth per day

Differences in new leaf growth per day between locations are presented in *figure 13a*. It can be seen that the natural shoots taken from Lac Cai and Sorobon grew a greater length in new leaf material than the shoots taken out of both research locations.

Leaf surface

In *figure 13b* we see the difference in leaf surface between the four sites. It is indicated in the figure that natural *T. testudinum* shoots had a bigger leaf surface (in Lac Cai and Sorobon) than the seagrass transplants in both research locations. A two-way ANOVA was used and no significant differences in leaf surface of *H. stipulacea* were found between both research locations and treatments.

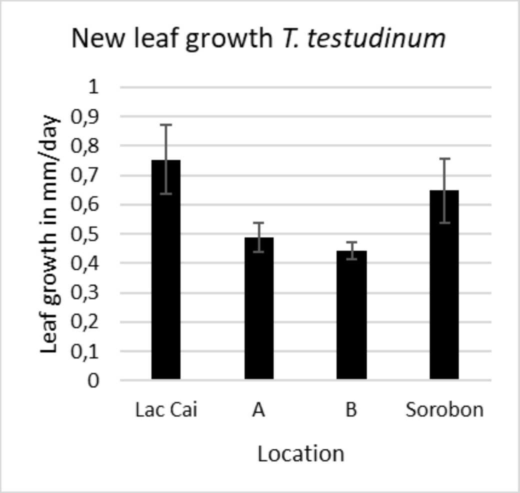

figure 13a: Growth of new leaf material in millimetres per day per site

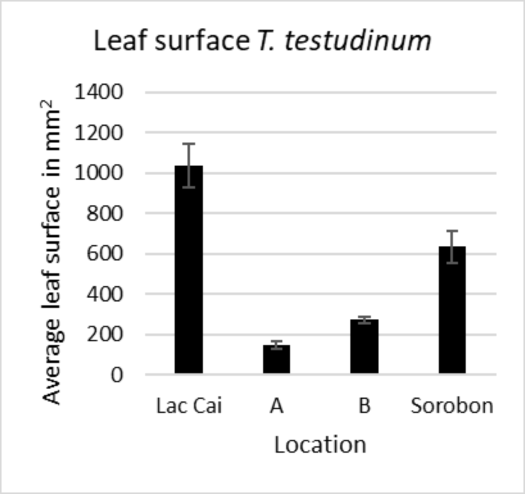

figure 13b: Average leaf surface in squared millimetres per site

Results of tested growth factors described above are summarized in table 1.

Table 1: Summary of statistics regarding tested growth factors per seagrass species

Factor	Seagrass species	Type of test	P value	Description
Fragment survival	- *T. testudinum* - *H. stipulacea* - Mono vs. Mix *T. testudinum* - Mono vs. Mix *H. stipulacea*	-ranked ANOVA -two-way ANOVA -ranked ANOVA -two-way ANOVA	-Interaction: p=0.06 -Treatments: p=0.00 -Interaction: p=0.02 -Treatments: p=0.01	-No significant interaction, however p value indicates possibility of interaction -Higher survival in control plots -Higher survival in BESE mixed plots and in control mono plots -Higher survival in control plots
Lateral expansion	- *T. testudinum* - *H. stipulacea* - Mono vs. Mix *T. testudinum* - Mono vs. Mix *H. stipulacea*	-two-way ANOVA & Welch t-test location B only -two-way ANOVA -ranked ANOVA -two-way ANOVA	-ANOVA: treatments: p=0.09, t-test: p=0.06 -Treatments: p=0.02 -Treatments: p=0.01 -Treatments: p=0.01, Monomix: p=0.03	-No significant differences between treatments for both tests, although p values indicate possible difference -Greater lateral expansion in control plots -Greater lateral expansion in control plots -Greater lateral expansion in control and mixed plots
Shoot growth	- *T. testudinum* - *H. stipulacea* - Mono vs. Mix *T. testudinum* - Mono vs. Mix *H. stipulacea*	-two-way ANOVA & t-test location B only -two-way ANOVA -two-way ANOVA -two-way ANOVA	-ANOVA: Interaction: p=0.03, t-test: p=0.01 -Treatments: p=0.06 -Interaction: p=0.01 -No significant differences, p>0.1	-Significant interaction, t-test location B shows higher shoot growth in control plots -No significant differences between treatments, although p value indicates possible difference -Significant interaction, higher control mono plots
Leaf productivity	- *T. testudinum* - Mono vs. Mix *T. testudinum*	-two-way ANOVA -two-way ANOVA	-Locations: p=0.02 -No significant differences, p>0.1	-Higher leaf productivity at location A

3.2 Environmental factors

The results of comparisons of different environmental factors are described below.

3.2.1 Wave action

Wave action was compared between both research locations. A significant difference in wave action between both locations was found (t-test, t(21.9)=9.5, p=0.00). This means differences in weight between location A were significantly bigger than at location B, which means wave action is higher at location A, shown in Annex 5H.

3.2.2 Bulk density sediment

Bulk density of the sediment was compared between both research locations. Performed t-test resulted in a significant difference in bulk density of the sediment between both research locations (t(3.5)=5.7, p=0.01). As shown in Annex 5I, bulk density of the sediment was higher at location A.

3.2.3 Water temperature

The water temperature was compared between both research locations. No significant difference in water temperature was found, (t-test, t(21.9)=-1.8, p=0.09). This can also be seen in the boxplot shown in Annex 5J. However, when only testing the first five days, temperature was significantly higher at location B (t(7.6)=-2.8, p=0.03).

3.2.4 Light intensity

Light intensity was compared between both research locations as well by using the hobo logger. No significant difference in light intensity was found, although it looked like light intensity was slightly higher in location B (t-test, (16.3)=-1.8, p=0.09). This can also be seen in the boxplot shown in Annex 5K. However, when only testing the first five days, light intensity was significantly higher at location B (t(5.0)=-5.2, p=0.00).

3.2.5 Bioturbation

The performed ranked ANOVA resulted in a significant difference in holes counted between locations (f(1)=25.2, p=0.00) as can be seen in *figure 14* below.

Sediment cores that were taken on both locations resulted in total in four mud shrimps (*Upogebia affinis*) caught at location B and at location A six unknown species of the class Polychaeta.

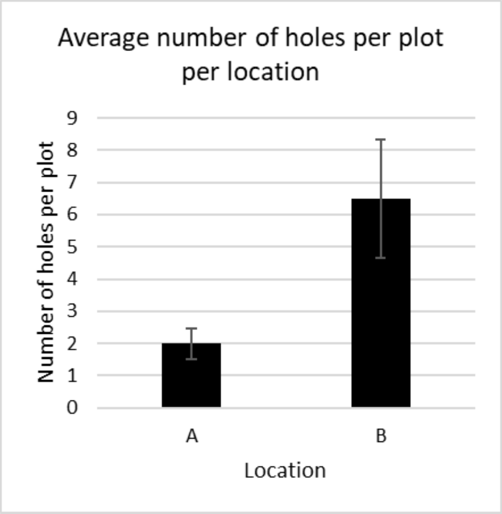

figure 14: Average number of holes per plot shown per location

3.2.6 Porewater

From porewater various aspects were measured, below the results of oxygen, salinity, phosphorus and nitrogen measurements are presented.

Oxygen

The performed two-way ANOVA showed a significant interaction between treatment and locations (f(1)=5.5, p=0.04), which is shown in *figure 15* below. In this boxplot it is shown that at location B there was a higher oxygen availability in the BESE plots while at location A there was a higher oxygen availability in the control plots. Other than that there is also a significant difference between both locations (f(1)=11.4, p=0.01), meaning there was a higher oxygen availability in the porewater at location B (*figure 15*).

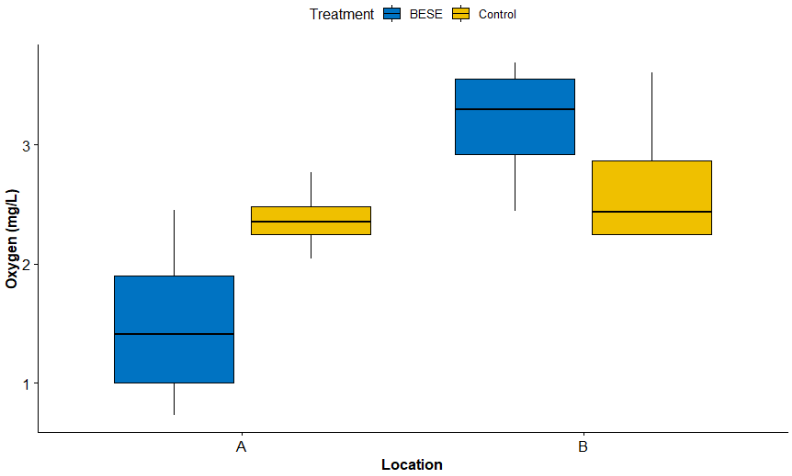

figure 15: Oxygen availability between locations and treatments

Salinity

The results of a two-way ANOVA showed there were no significant differences in salinity in porewater between locations and treatments.

Phosphorus

A ranked ANOVA was performed which showed that there were no significant differences in phosphorus concentration in porewater present between locations and treatments.

Nitrogen

There was also no significant difference in nitrogen concentrations found between locations and treatments.

3.2.7 Sediment mobility

As explained in the methods chapter, sediment mobility was measured in three different ways. Firstly, the differences between begin vs. end measurement of the distance from the top of the sediment pin to the sediment were compared. Then, the maximum distances between the top of the sediment pin to the sediment were compared per plot. Lastly, sediment mobility was measured by looking at the average difference between the ring and the sediment per plot. There were no significant differences found in sediment mobility between locations and treatments by using either of these three methods. For all three datasets a ranked ANOVA was performed.

3.2.8 Surface water samples

Oxygen

The performed Welch t-test showed that there was no significant difference in dissolved oxygen availability in the water between both research locations.

Temperature

No significant difference in temperature between both locations was shown by the performed t-test.

Salinity

No significant difference in salinity between both research locations was shown by the performed t-test.

In table 2 a summary is made of the results of all the statistical tested environmental factors described above.

Table 2: Summary of statistics regarding tested environmental factors

Factor	**Type of test**	**P value**	**Description**
Wave action	t-test	0.00	Significant higher wave action at location A
Sediment bulk density	t-test	0.01	Significant higher sediment bulk density at location A
Water temperature (HOBO)	t-test	0.09	No significant difference in water temperature, although an indication there might be a difference with higher temperatures at location B
Light intensity (HOBO)	t-test	0.09	No significant difference in light intensity, although an indication there might be a difference with higher light intensity at location B
Bioturbation	Ranked ANOVA	Locations: 0.00	Significant higher number of bioturbation holes at location B
Dissolved oxygen in porewater	Two-way ANOVA	Interaction: 0.04 Locations: 0.01	Significant interaction, higher oxygen availability in BESE plots at location B while higher oxygen availability in the control plots at location A, general higher oxygen availability at location B
Presence of: **Salinity** **Phosphorus** **Nitrogen** in porewater	Two-way ANOVA, ranked ANOVA, ranked ANOVA	p>0.1 for all	No significant differences in salinity, phosphorus and nitrogen between locations
Sediment mobility	Ranked ANOVA	p>0.1	No significant differences in sediment mobility between locations and treatments
Surface water samples (oxygen, salinity, temperature)	Welch t-test, t-test, t-test	p>0.1	No significant differences in oxygen availability, salinity and temperature of surface water samples between locations

4. Discussion

4.1 Impact of sediment stabilizing root mats on the native *T. testudinum* restoration success

In general it was expected to find a positive relationship between the use of BESE sheets and *T. testudinum* restoration success, especially in areas with high hydrodynamics. This was expected due to the sediment stability that BESE sheets provide which enhances settlement of *T. testudinum*. It was expected that fragment survival of *T. testudinum* would be higher in BESE plots, since BESE structures provide sediment stability (*BESE Products*, 2021). Together with this a higher shoot growth was also expected, since it was expected that more fragments would survive in BESE sheets. For the lateral expansion and leaf productivity no specific differences were expected.

In general it is found that the impact of BESE structures on seagrass growth differs depending on present environmental conditions. In this case especially different hydrodynamics show a difference in effect of these stabilizing root mats on *T. testudinum* growth. No significant differences between treatments and locations were found in fragment survival and lateral expansion. However a significant difference was found in shoot growth between treatments in location B and a significant difference in leaf productivity was found between locations.

The significant higher leaf productivity at location A compared to location B can be explained by the difference in hydrodynamics. By using plaster sticks it was found that hydrodynamics significantly differ between both research locations, with higher hydrodynamics present at location A. In a study done by Fonseca & Kenworthy (1987), a positive relationship was found between current velocity and leaf productivity. Current flow is expected to reduce the diffusion boundary layer which enhances nutrient uptake at the leaf surface (Fonseca & Kenworthy, 1987). Since at location A a higher wave action was present, this could explain the higher leaf productivity at this location.

According to Koch (1999), favourable growth conditions for *T. testudinum* are linked to nutrient availability. This in turn is linked to hydrodynamic conditions since waves and currents cause sediment to suspend, thereby causing release of nutrients (Koch, 1999). A suspected interaction between nutrient uptake and availability and hydrodynamic conditions is expressed by Koch (1999) and Fonseca & Kenworthy (1987). No difference in phosphorus and nitrogen concentrations between locations and treatments were found. This could however be due to accuracy of the nutrient kit that is used. Values varied between timepoints of measurements and measured plots and ultimately no patterns could be discovered. However, this could also be an accurate representation of nutrient concentrations at both locations which would mean difference in leaf growth is not linked to nutrient concentrations. To ensure this, more in depth nutrient testing is advised.

Other important factors influencing leaf growth are light availability, temperature, salinity, fish grazing and oxygen availability. No significant differences in light and nutrient availability, temperature and salinity were found between locations and are thus not assumed to cause the difference in leaf productivity (Borum et al., 2004). Fish grazing was not evaluated, this is something that could be done in the future. However from observations during monitoring, grazing was not suspected to cause major impacts on growth of leaves. Oxygen availability in porewater differed significantly between research locations, however this was higher in location B and is thus not contributing to the higher leaf productivity at location A. The used method to calculate leaf productivity could also have influenced the outcome. The total new leaf length of a shoot was divided by the total original and new leaf length, which was then multiplied by 100%. The result is called the percentage of new leaf growth. Since location A was implemented later, it could be that shoots in general were small and therefore the proportion of new leaf length increased more than the proportion of new leaf length out of the total leaf length of *T. testudinum* shoots present in location B. When comparing the new leaf growth per day between both research locations and natural shoots at both Sorobon and Lac Cai, it is indicated that natural shoots in both natural locations have a higher growth per day (*figure 13a*). However, the new leaf growth of all these four locations are not statistically tested against each other (only both research locations are tested against each other). This could be explained by the total leaf length and width which is higher for these natural shoots. The method taking into account total leaf length was used to compare leaf productivities, since this was deemed to give a more inclusive representation.

Although not significant, there is an indication of a possible interaction in fragment survival between location and treatment. Results shown in *figure 8* indicate a possible difference in fragments survival between treatments at location A. This was expected and could be explained by the difference in wave action between both locations. Since wave action is significantly higher at location A, the probability for fragments to wash out is higher in this area (Schanz & Asmus, 2003). When hydrodynamics increase, sediment stability in general tends to decrease (Ziegler, 2002). BESE mats provide sediment stability (*BESE Products*, 2021; Temmink et al., 2020), however in control plots this was not provided. This explains the lower fragment survival in control plots compared to BESE plots at location A. The small difference in fragment survival between treatments in location B can be explained by the weaker hydrodynamics present at this location which reduces the importance of the use of BESE mats to create sediment stability and enhance settlement. A possible reason for the difference in fragment survival between treatments at location A not being shown could be the difference in fragment survival between control plots. In two of the control plots all fragments washed away, in the third control plot only one fragment remained, while in the fourth control plot five fragments remained. This caused a high variation in fragment survival within control plots.

The lost fragments of *T. testudinum* in the control plots at location A led to a lack of data which makes it difficult to compare lateral expansion between locations and between treatments within location A. Therefore, difference in lateral expansion of *T. testudinum* in location B is investigated. A possible difference in lateral expansion between BESE and control plots is indicated in *figure 9*. In control plots lateral growth seems bigger. This can possibly be explained by the structure of the BESE mats. Since the *T. testudinum* fragments are clicked in between two BESE sheets, the rhizomes need to find their way through the openings in these mats to be able to grow horizontally. Lateral expansion of *T. testudinum* fragments can thus be obstructed by the lattice of the BESE mats. There is a strong relation between rhizome biomass and shoot density, which implies that a hampered lateral growth could also influence shoot growth negatively (Gallegos et al., 1993). When looking at shoot growth, the interaction between location and treatment shows that the effect of a treatment on shoot growth differs between locations. There is no significant difference in shoot growth between treatments at location A. It is difficult to draw conclusions since at location A shoot growth was highly influenced by the lack of remaining fragments. When fragments are gone after settlement, shoot growth will automatically be zero which happened for two complete control plots at location A. However, there is a significant difference between treatments when looking at location B only, namely a higher number of added shoots in control plots as compared to BESE plots (*figure 10*). This could mean that indeed due to the structure of the BESE mats lateral growth is reduced, leading to a reduction in shoot growth as well. However, this was not tested statistically and can thus not be stated with certainty. Additionally, difference in shoot growth between both locations is difficult to compare since the amount of time the fragments were able to grow, differs per location. The fragments at location A were planted roughly one month after the implementation of the fragments at location B.

Lastly, bioturbation, which is significantly higher in location B, seems to influence the seagrass restoration success in this location. Bioturbation can negatively affect both *T. testudinum* and *H. stipulacea* by burrial and smothering of the seagrass as well as by mound building activities (Townsend & Fonseca, 1998; Valentine et al., 1994) which was observed in both control and BESE plots. In control plots fragments appeared to be buried by mounts of sediment and fragments disappeared in the surroundings of holes and mounts. Some fragments of *T. testudinum* in BESE sheets became exposed again presumably due to bioturbation activities which caused sediment removal in parts of BESE sheets. When these fragments were exposed signs of grazing were visible on shoots and in some cases on the rhizome as well. When *T. testudinum* fragments stayed exposed and uncovered, this mostly resulted in dying or disappearing of the fragment. However, to be able to draw conclusions out of these observations, broader research into bioturbation and the effects of this on the success of seagrass restoration projects involving BESE mats is recommended.

4.2 Impact of sediment stabilizing root mats on the invasive *H. stipulacea* restoration success

It was a slightly difficult to set expectations for the relationship between the use of BESE sheets and restoration success of *H. stipulacea* since there was no information about this yet. It could be that there would also be a positive relationship between the use of BESE sheets and *H. stipulacea* restoration success due to the provided sediment stability and the structure of BESE sheets. The sediment stability and structure of BESE sheets were expected to enhance settlement and provide substrate for settlement of new *H. stipulacea* fragments.

In general it is found BESE structures negatively influence growth of *H. stipulacea*. Significant differences between treatments and locations were found in both fragment survival and lateral expansion. An indication for higher shoot growth in control plots was found.

The significant higher survival of *H. stipulacea* fragments and rhizome growth in control plots could have multiple reasons. It could be that reimplementing *H. stipulacea* fragments in BESE sheets might have caused stress and or damage to these fragments. Fragments needed to be placed between the two BESE sheets and therefore needed to be pushed and pulled through the mats. As a consequence of *H. stipulacea* being a small seagrass species with smaller leaves, rhizomes and roots compared to *T. testudinum*, fragments have a low tolerance to disturbances (Duarte et al., 1997; Malm, 2006; Winters et al., 2020). This makes *H. stipulacea* fragments prone to stress caused by the implementation process. As a result, it is possible that fragments (partly) died or experienced a reduction in growth. Another reason could be a higher chance of burial in BESE plots. This could be explained by reviewing the reimplementation method. Sedimentation occurred on BESE and control plots after the first implementation of fragments. Some BESE sheets were covered by sediment when *H. stipulacea* fragments were implemented for the second time. Fragments needed to be placed in between the BESE sheets and were covered again with sediment by currents and waves after reimplementation. Fragments in control plots did not have to be placed between buried sheets and were therefore not dug deep into the sediment. This implementation difference could have caused a difference in fragment survival and rhizome growth between treatments. Smaller seagrass species such as *H. stipulacea* are highly sensitive to burial and can be reduced greatly by only slight burial (Cabaço et al., 2008; Duarte et al., 1997). Fragment burial could have caused smothering of the fragments and thus (partial) die-off of fragments. Besides this and similarly to the fragments of *T. testudinum*, a contributing factor could be the complication of growth due to the structure of BESE mats. However, *H. stipulacea* is a smaller seagrass species than *T. testudinum*. Rhizomes of *H.*

stipulacea have a smaller diameter, namely 0.5-2.0 mm (Den Hartog, 1970; Willette & Ambrose, 2009), while rhizomes of *T. testudinum* have a diameter of about 14.8 mm (Cabaço et al., 2008). Therefore, it is unlikely that BESE sheets cause a decrease in rhizome growth. Although not significant, a difference in shoot growth was observed between treatments for *H. stipulacea* plots. More shoots have grown in control plots than in BESE plots. This is expected to be explained by the same reasons as mentioned above.

No new fragments settled on the BESE structures, neither on control plots. This could mean that BESE sheets do not indirectly facilitate growth of the invasive *H. stipulacea.* However, since also no new fragments settled on the control plots, this conclusion can not be drawn with certainty. Therefore further research is suggested, in this case a longer monitoring period.

4.3 Impact of restoring monospecific vs. mixed seagrass species

Fragment survival was also tested comparing treatments and plots with either only *T. testudinum* (mono) or *H. stipulacea* (mono) and plots consisting of both *T. testudinum* and *H. stipulacea* fragments (mixed).

For both the native *T. testudinum* and the invasive *H. stipulacea* a higher lateral expansion was found in control plots as explained above. For *H. stipulacea* also a significant difference in lateral growth between mixed plots and mono plots is found, namely a higher lateral expansion in mixed plots. This can be explained by the lack of data available about mixed plots since fragments washed away. Only one mixed BESE plot and one mixed control plot still contained *H. stipulacea* fragments that could be measured. Therefore, we can conclude that this test outcome is based on an insufficient amount of data and further research is needed to be able to draw conclusions about differences in lateral growth between mono and mixed plots.

For *T. testudinum* no significant difference in fragment survival between treatments was detected, however there is an indication that possibly a higher survival occurs in BESE plots. Explanations for this are already given in the *T. testudinum* section. The significant interaction, visualised in Annex 5B, shows higher fragment survival in mixed BESE plots than mono BESE plots, while in control plots fragment survival of mono plots is higher than in mixed plots. The higher survival in mono control plots could possibly be explained by the positive effect of higher seagrass densities on sediment stability (Suykerbuyk et al., 2016). Since *T. testudinum* is a bigger seagrass with a bigger rhizome and roots deeper than *H. stipulacea* a higher root density and sediment stability could be provided by mono plots which enhances fragment settlement (Suykerbuyk et al., 2016; Temmink et al., 2020; van Katwijk et al., 2016; Willette & Ambrose, 2009). BESE mats provide sediment stability (*BESE Products*, 2021; Temmink et al., 2021) which could be the reason that this trend is not seen in BESE plots. For the invasive *H. stipulacea* there is only a significant difference in fragment survival between treatments, with a higher fragment survival in control plots. An explanation for this is already given in the section '*H. stipulacea*' above. Between mono and mixed plots no difference in fragment survival is found. The seagrass *H. stipulacea* roots shallow, unlike *T. testudinum* does and thus does not provide the same amount of sediment stability (Den Hartog, 1970; Winters et al., 2020). Therefore, the positive effect of a higher seagrass density on sediment stability could be dampened in the case of *H. stipulacea* (Christianen et al., 2013; Smulders et al., 2017; Vonk et al., 2015).

For shoot growth of *T. testudinum* a significant interaction was found, showing that in BESE plots mono *T. testudinum* shoots grow slower than shoots in BESE mixed plots, while in mono control plots *T. testudinum* shoots grow faster than in mixed control plots. Mainly in control plots a difference in shoot growth is visible with a higher shoot growth in mono plots (*figure 12*). This could be related to fragment survival which was higher in mono control plots as described above. For the invasive *H. stipulacea* no significant differences in shoot growth are found between mono and mixed plots, which could be explained by the fact that there is no difference in survival between mono and mixed plots either. The lacking difference of shoot growth between BESE and control plots is difficult to explain. Although it is indicated that an insufficient amount of data is available, since fragments washed away.

There is no difference in leaf productivity between treatments and mono and mixed plots for the native *T. testudinum*, which fits with previous results stating there is only a significant difference between research locations and not within locations.

5. Conclusion

In this project we investigated how seagrass restoration using biodegradable sheets influenced the balance between native and invasive seagrass. During this research the effect of BESE structures on the native *T. testudinum*, the invasive *H. stipulacea* and a mix of both species was researched at two locations. These locations significantly differed in wave action.

BESE products are useful products to use when restoring the native seagrass *Thalassia testudinum*. They are especially of use in environments with high wave action and currents due to their ability to provide sediment stability. Next to that, fragments are hold in place which prevents washing away and enhances settlement. However, for the long-term BESE sheets possibly negatively affect seagrass growth. It is speculated that BESE sheets possibly interfere with rhizome growth. For the seagrass species *Halophila stipulacea* BESE products do not provide advantages in terms of growth. Fragments of *H. stipulacea* are fragile and possibly suffer from stress when implementing in between BESE sheets. Other than that burial affects these fragments. Higher growth of *T. testudinum* in monospecific control treatments than mixed control treatments could be explained by higher sediment stability provided in monospecific control treatments due to a higher seagrass density. However, especially for *H. stipulacea*, low fragment survival reducing the sample size may have also influenced the differences between monospecific and mixed treatments. Therefore, it is suggested to do further research. An experiment with more replicates focusing on growth in multiple different hydrodynamic conditions could give more insights.

Next to hydrodynamics affecting seagrass growth and the effectiveness of BESE products, also bioturbation is likely to negatively influence seagrass expansion. Knowledge about the effects of bioturbation in combination with BESE products on seagrass restoration would be valuable to obtain and therefore further research specifically on the effects of bioturbation on the impact of BESE products in combination with seagrass growth is advised.

It can be concluded that BESE products positively influence restoration of the native *T. testudinum*. And may positively influence the balance between native and invasive seagrass. These products enhance the initial survival of *T. testudinum* and do not positively influence survival of *H. stipulacea*. However, it is indicated that BESE products possibly hamper rhizome growth which reduces long-term growth of *T. testudinum*. Future research into rhizome growth of *T. testudinum* in combination with BESE plots is needed to determine this with certainty. This could be done by repeating the experiment with more plot replicates and rhizomes so that a sufficient amount can be taken out to measure. A different method could perhaps be used in this experiment to ensure fragments stay in place and do not wash out. It would be valuable to see if in that case a significant difference exists in lateral expansion and shoot growth between treatments in both locations.

Furthermore, it would be interesting to set up an experiment to test the correlation of hydrodynamics and the importance of using BESE sheets for seagrass restoration. This could be done by testing fragment survival in a wider range of different hydrodynamic conditions.

To conclude, the effectiveness of BESE products depends on the seagrass species and environmental conditions such as hydrodynamics. In areas with high hydrodynamics BESE product area a useful restoration tool since they can provide the necessary sediment stability for *T. testudinum* fragments to settle. In areas with low hydrodynamics the positive effect of BESE sheets is minor. However, short and long term effects of BESE products differ as stated before. In areas with invasive seagrass, BESE products can serve as a tool to shift the balance towards native seagrass species. Therefore, BESE products can be seen as a tool to interfere and steer competition between native and invasive species.

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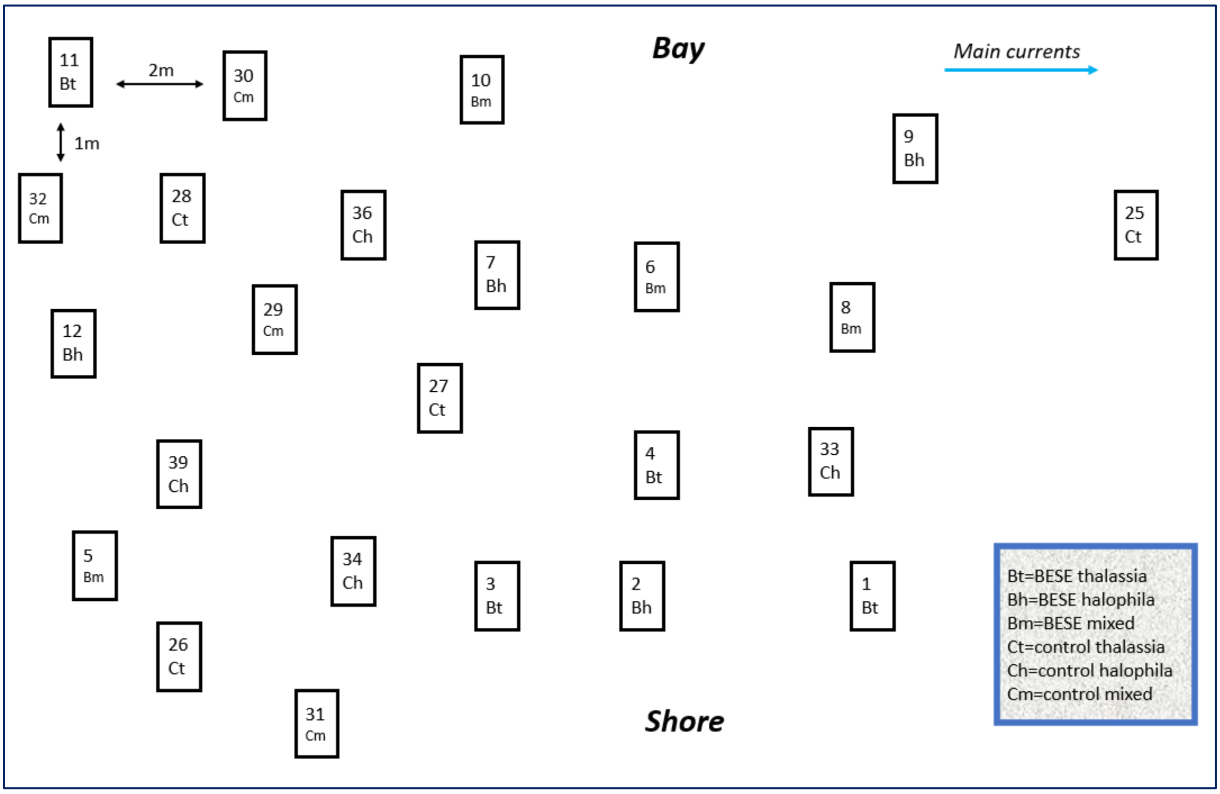
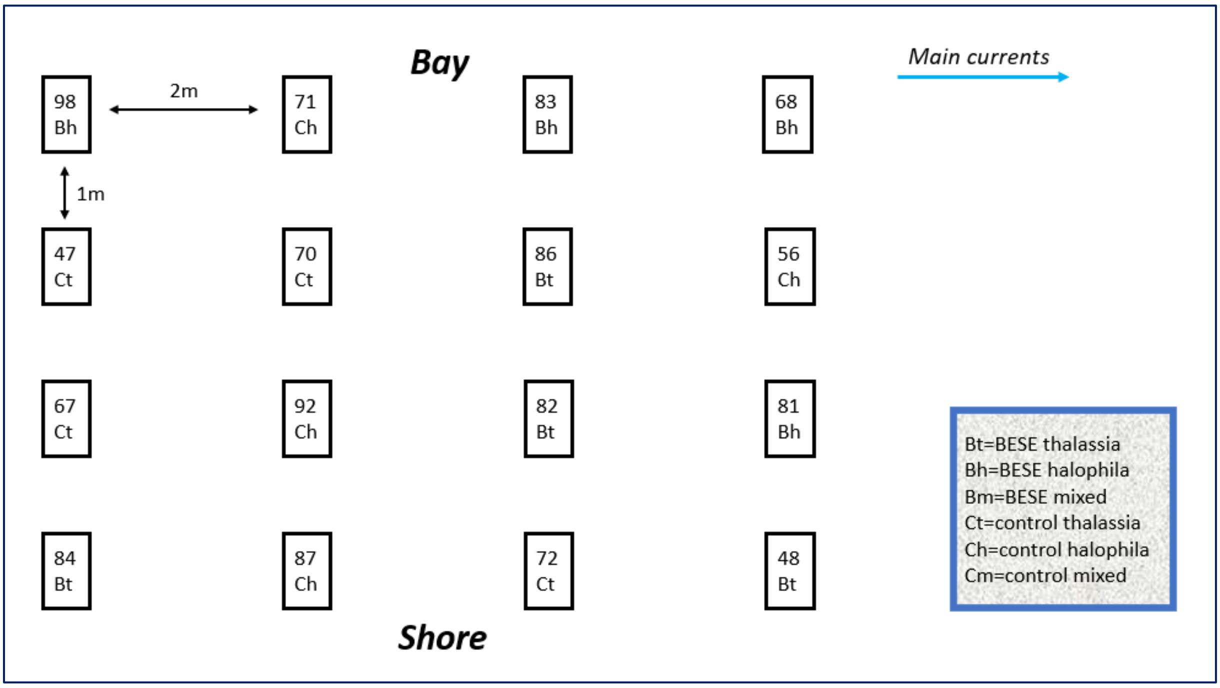
Annexes

Annex 1: Map of location B



Map location B, numbers are plot numbers, abbreviations are described in the right lower corner

Annex 2: Map of location A



Map of location A, numbers are plot numbers, abbreviations are described in the right lower corner

Annex 3: Table implementation dates

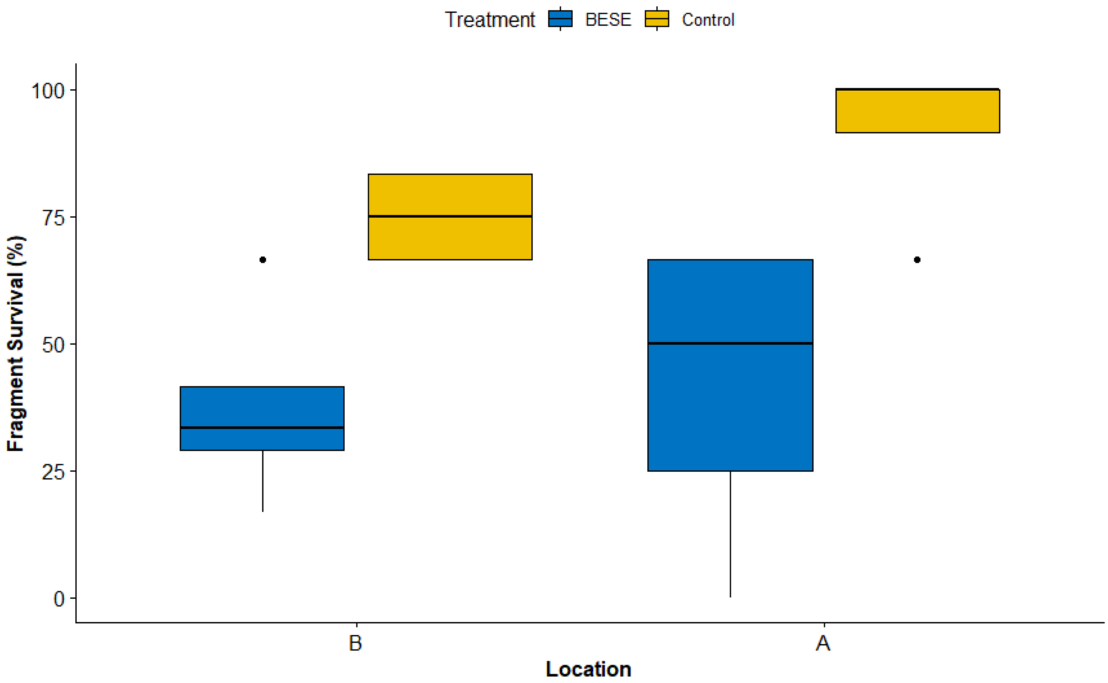
Implementation, settlement and take out dates per location, seagrass species and treatment

Location	**Seagrass species**	**Treatment**	**Date implementation**	**Date settlement**	**Date out**
B	*T. testudinum*	BESE Control	10-nov-2021 15-nov-2021	6-dec-2021	31-jan-2022
	H. stipulacea	BESE & Control	12-jan-2022	17-jan-2022	31-jan-2022
	Mix *T. testudinum*	BESE Control	11-nov-2021 15-nov-2021	6-dec-2021	31-jan-2022
	Mix H. stipulacea	BESE & Control	12-jan-2022	17-jan-2022	31-jan-2022
A	*T. testudinum*	BESE & Control	15-dec-2021	3-jan-2022	31-jan-2022
	H. stipulacea	BESE & Control	12-jan-2022	17-jan-2022	31-jan-2022

Annex 4: Protocol hole-punch method

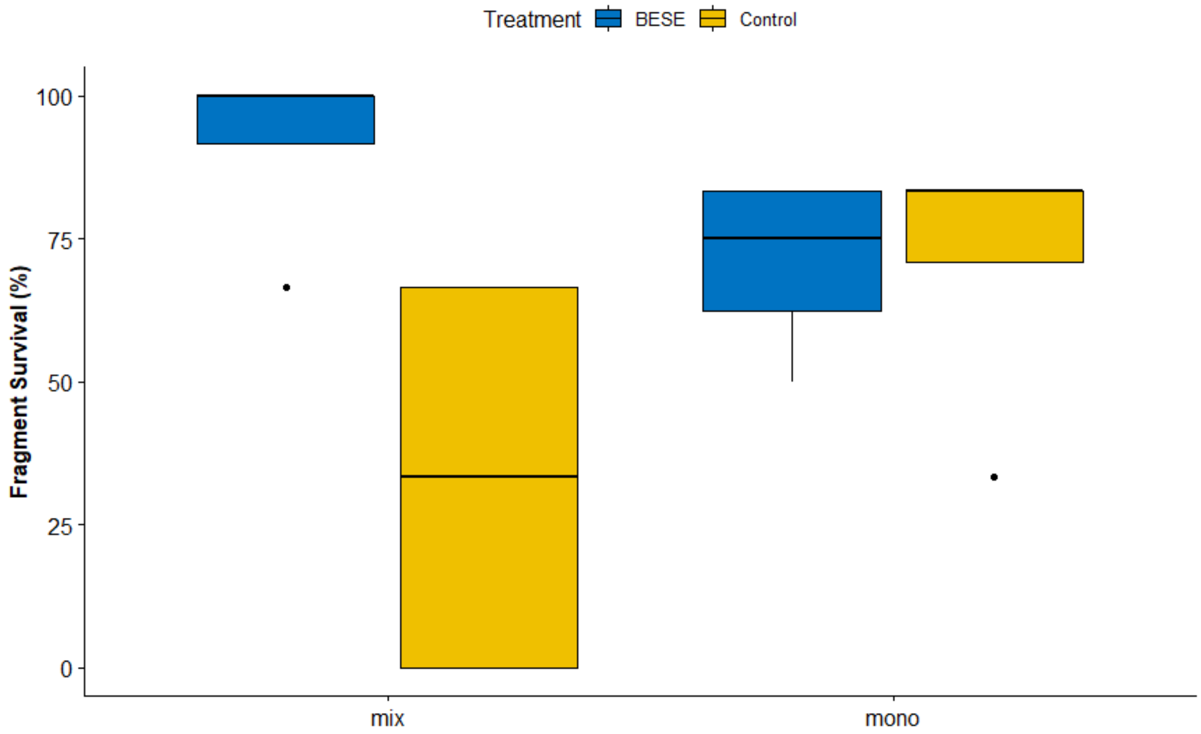
When using the hole-punch method to determine leaf productivity, a hole is pierced through all the leaves of a shoot with a small needle at the place where the colour changes from white to green. In this case holes were made in all shoots of two fragments of each plot including *T. testudinum* at both research locations. Other than that natural seagrass was also punched to be able to compare leaf productivity. This was done by using a ring with a diameter of 16 cm. This ring was placed on two random spots with natural growing seagrass in Lac Cai close to location B. All the shoots in this ring were punctured and this area was marked with self-made little flags existing out of small wooden pins with folded pink tape on the top. These were used to be able to find the pierced shoots back. Also in Sorobon, the location where seagrass fragments were harvested for the experiment, two rings were placed and the process was repeated. Eleven days after piercing the holes, all the seagrass fragments and shoots in Lac Cai were collected and taken out. In Sorobon the punctured shoots were taken out after fifteen days, this was done later due to time lack to immediately process all fragments. When taken out the fragments were labelled with tape of a specific colour which stands for their position in the plot. These fragments were put into a marked ziplock bag with some water inside to keep them in a good condition. All the bags were put into a cool box with seawater inside and transported home. At home the processing started which included measuring the width, length of new growth and length of old growth per leaf per shoot and noted down in an excel sheet.

Annex 5A: boxplot fragment survival *H. stipulacea*



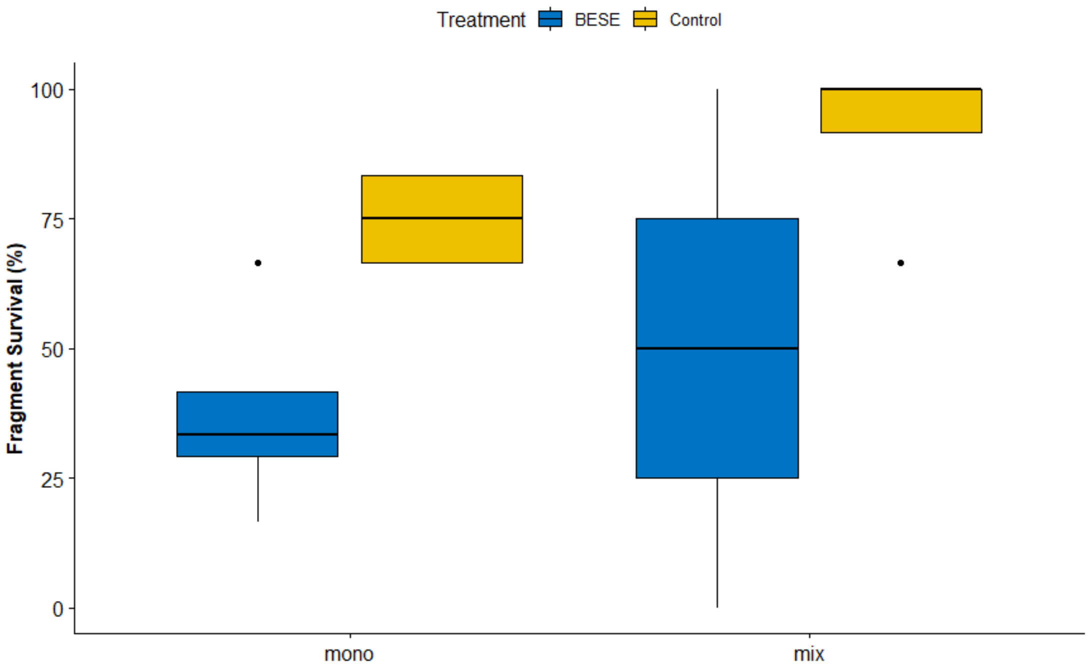
Boxplot showing significant difference in fragment survival of H. stipulacea between treatments, in this case higher fragment survival in control plots

Annex 5B: boxplot fragment survival *T. testudinum* mix vs. mono



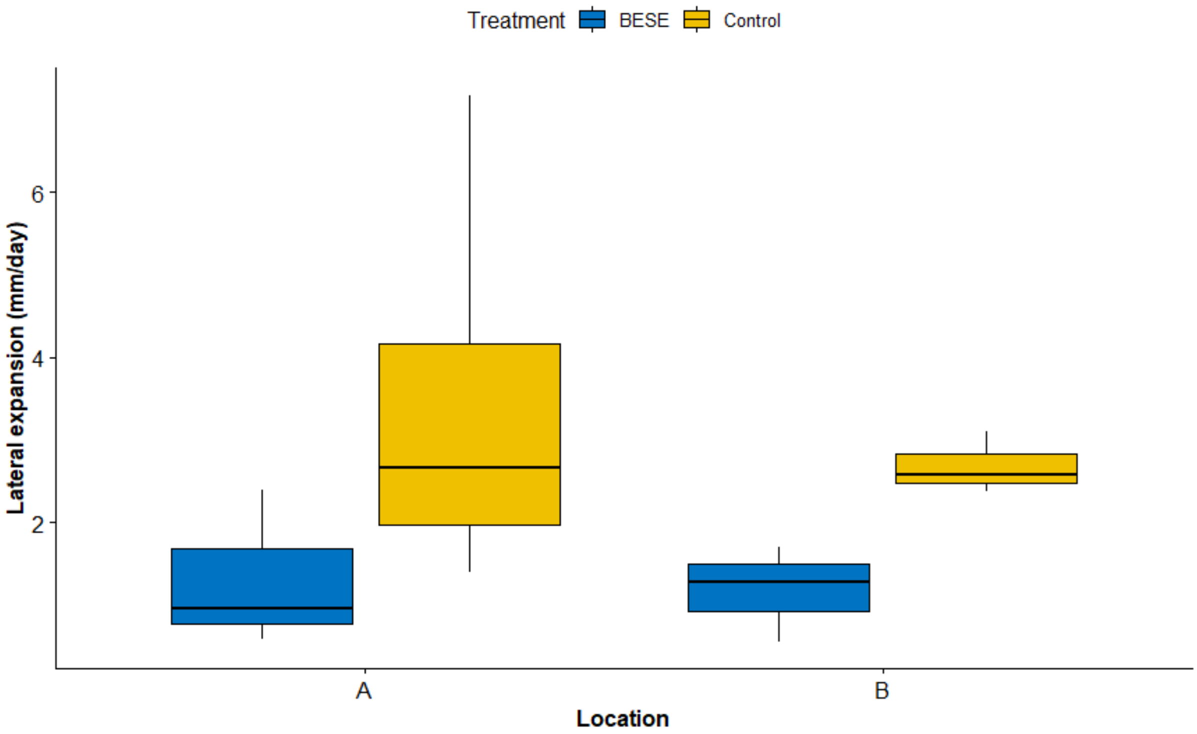
Boxplot showing significant interaction of fragment survival between treatments and mono/mix plots, in this case higher fragment survival in mono control plots and mixed BESE plots

Annex 5C: boxplot fragment survival *H. stipulacea* mix vs. mono



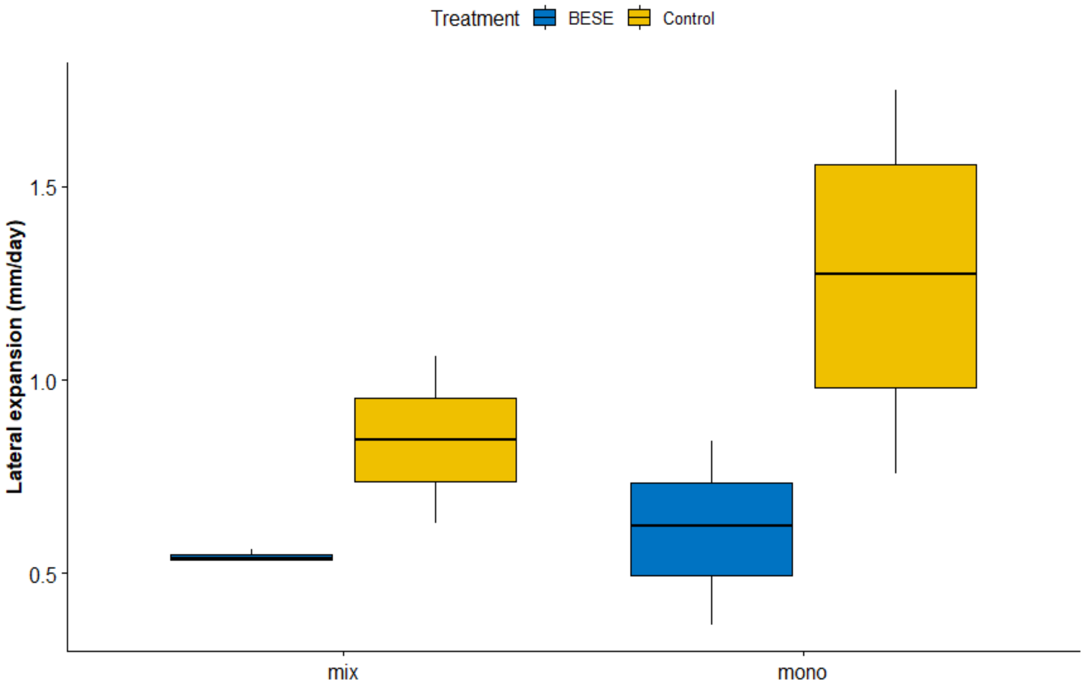
Boxplot showing significant difference in fragment survival between treatments, in this case higher fragment survival in mix and mono control plots

Annex 5D: boxplot lateral expansion *H. stipualcea*



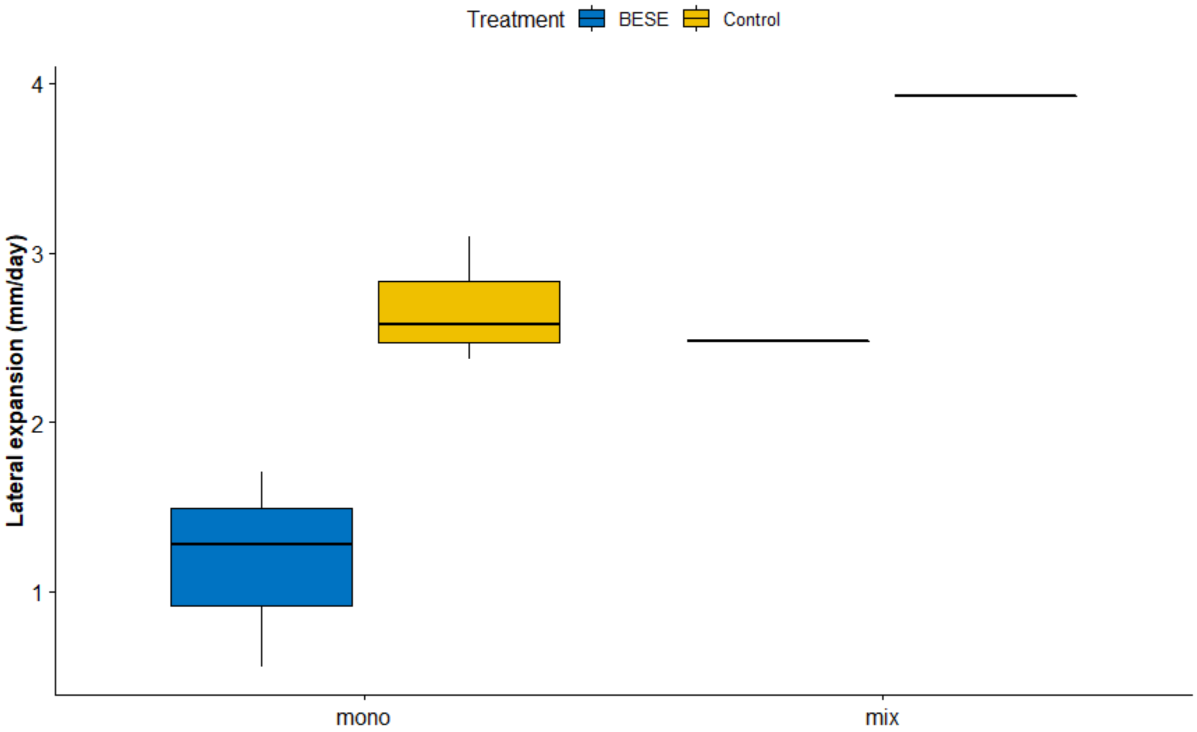
Boxplot showing significant difference in lateral growth of H. stipulacea between treatments. In this case faster lateral growth in control plots

Annex 5E: boxplot lateral expansion mix vs. mono *T. testudinum*



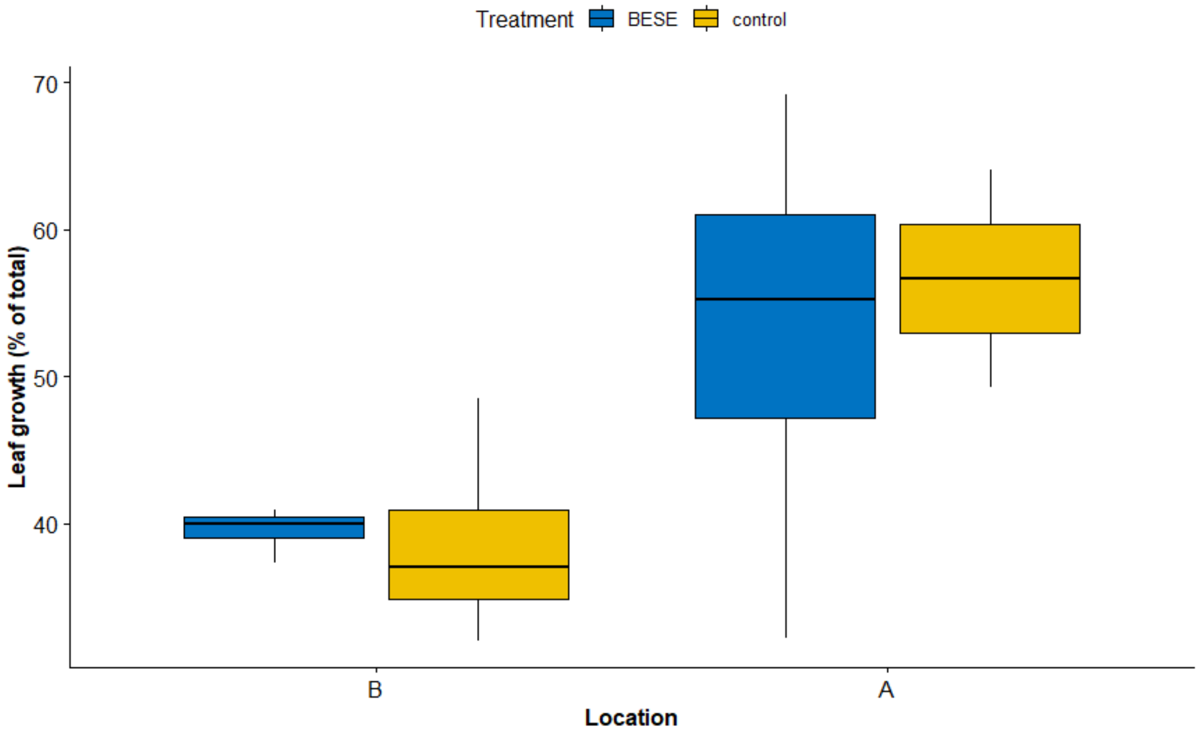
Boxplot showing significant difference in lateral growth of T. testudinum between treatments, in this case faster lateral growth in control plots

Annex 5F: boxplot lateral expansion mix vs. mono *H. stipulacea*



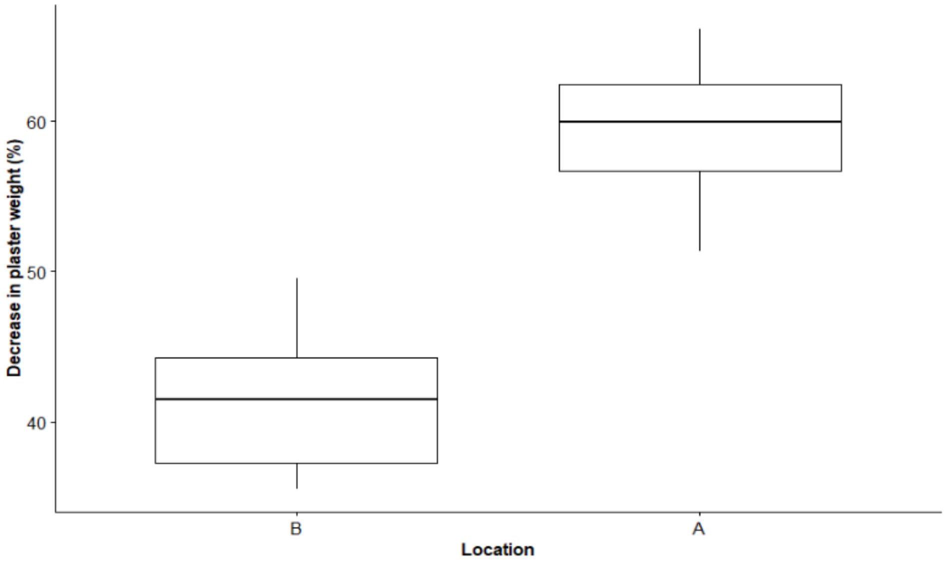
Boxplot showing significant difference in lateral growth of H. stipulacea between treatments and mono/mix plots, in this case faster lateral growth in control plots and in mixed plots

Annex 5G: boxplot leaf productivity



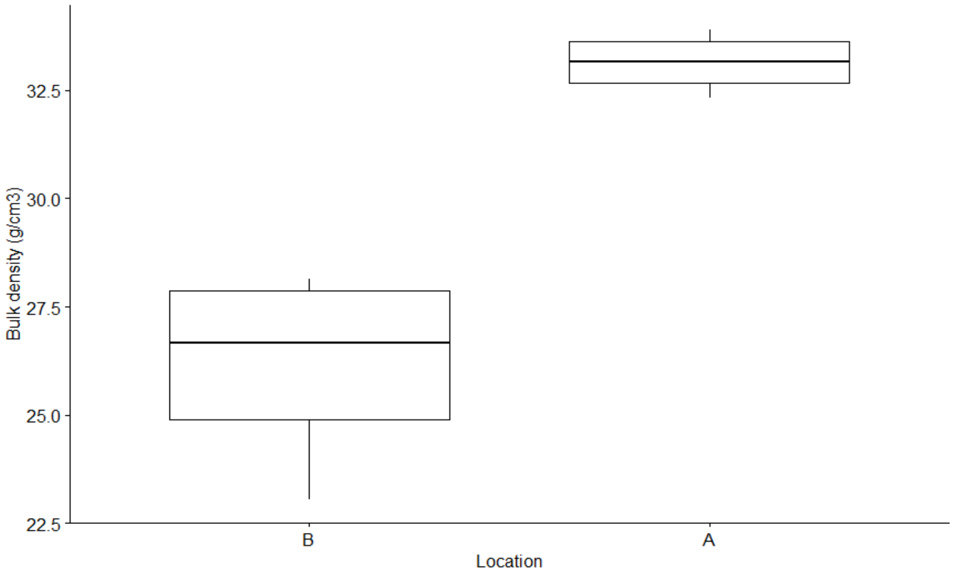
Percentage of new leaf growth out of total leaf length shown per location and treatment

Annex 5H: boxplot wave action between locations



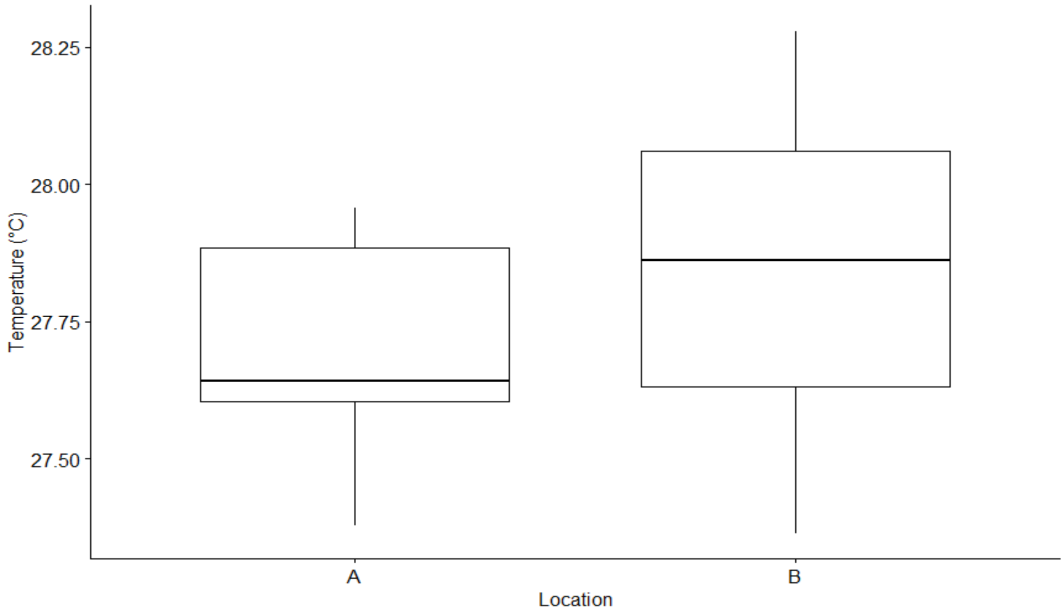
Boxplot showing significant difference in wave action between locations, in this case higher wave action at location A (a higher change in plaster weight induces more wave action)

Annex 5I: boxplot bulk density of the sediment between locations



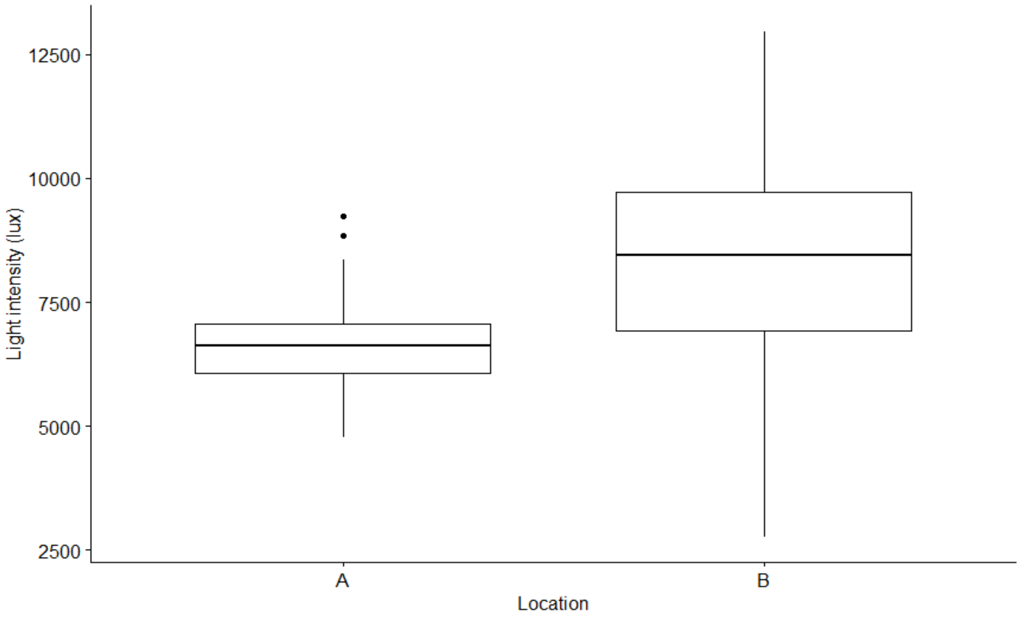
Boxplot showing significant difference in bulk density of the sediment between locations, in this case sediment has a higher bulk density at location A

Annex 5J: Boxplot temperature between locations



Boxplot showing difference (although not significant) in water temperature between sites, it is indicated that water temperature might be slightly higher at location B

Annex 5K: boxplot light intensity between locations



Boxplot showing difference (although not significant) in light intensity between locations, it is indicated that light intensity might be slightly higher at location B