UNIVERSITY OF GOTHENBURG

DEPARTMENT OF MARINE SCIENCES

EXPLORING FERTILIZATION STRATEGIES FOR *ZOSTERA MARINA* GROWTH: ORGANIC VS. INORGANIC FERTILIZER

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Picture 1: Photograph of the six shoots in one of the experimental mesocosms

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A popular scientific summary

Nurturing seagrass for a sustainable future

In a changing world, where people are getting increasingly more affected by human-induced climate change and environmental decline, the efforts to reduce the damages done both on the global scale, as well as locally, are becoming increasingly important before we reach a point of no return. One piece of the puzzle could be utilizing the seagrasses which help in a multitude of ways, such as excelling at capturing carbon from the atmosphere and from the particles which runoff from land, as well as increase the health of coastal fisheries. Unfortunately, human activities such as boating, introducing non-native species, construction, and other ways of decreasing the light available to these plants has caused a catastrophic loss globally.

Our research explores a fascinating new nutrient solution with the hope of increasing the growth of these vital seagrass ecosystems, for a better restoration potential and a greater burial. The key to this endeavour? Organic fertilizer, specifically arginine, known for its unique ability to enhance the root growth, and adhere to sediment, potentially curbing any side effects such as eutrophication. Our research delves into the potential of this organic fertilizer, comparing it to a traditional inorganic counterpart with the help of growing the seagrass species “eelgrass” in aquaria. We employed a variety of tools such as modern digital measuring tool ImageJ and traditional instruments such as vials and scales to shed light on the question of organic nutrient as a potential eelgrass growth-booster.

A promising path for eelgrass

The results suggest arginine as a promising nutrient source, significantly boosting the eelgrass’s root count. While other aspects of the plant’s structure remain indifferent to the inorganic fertilizer and control, the root number is particularly pronounced after a 6-week period. These findings underscore the potential of organic fertilizers for eelgrass restoration, although practical applications may require further investigation. Primarily, previous research suggests that during restoration, transplanted eelgrass is strong enough to survive harsh environmental conditions after a mere 10 days. However, further research is needed, especially with the context of mitigating climate change.

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Abstract

Seagrass species provide vital ecosystem services such as habitat provision and carbon sequestration. Over the last few decades, seagrass species, such as *Zostera marina* (commonly known as eelgrass) meadows, have experienced a vast decline, with a reduction close to 60% on the Swedish west coast. This paper investigates the use of arginine as an organic fertilizer to promote growth, particularly in terms of root development as has been shown in terrestrial studies, of the *Z. marina* within a mesocosm experiment, and comparing it to a traditional inorganic fertilizer. The objective is to increase the resilience and growth of *Z. marina*, especially during the growing season, and for restoration efforts, as replanted eelgrass shoots are potentially vulnerable during higher-than-expected energy events, caused by inadequate sediment anchorage.

After a period of six weeks, a significant increase in the number of roots was observed in the organic nutrient treatment compared to both the control and the inorganic fertilizer. For all other sampling times and measured morphological variables, no significant difference between any of the treatments occurred.

These results imply that organic fertilizers, such as arginine, can serve as a more efficient nitrogen source for seagrass species, especially increasing the root count. However, further research is required to assess the applicability of organic fertilizers as effects are only seen after a period of 6 weeks, and during restoration efforts eelgrass is typically deemed strong enough 10 days following replantation.

Introduction

As the urgency of mitigating climate change intensifies carbon sinks play an increasingly crucial role to capture anthropogenic carbon emissions (IPCC, 2022). Seagrass meadows possess a large capacity to sequester carbon, to stabilize the sediment, actively bury carbon through their extensive belowground root-rhizome system, as well as capture allochthonous carbon by particle trapping (Kenworthy & Thayer, 1984; Björk *et al*., 2008; Hendriks *et al*., 2008; Kennedy *et al*., 2010). Despite significant losses (Short & Wyllie-Echeverria, 1996; Baden *et al*., 2003; Waycott *et al*., 2009; Dunic *et al*., 2021), recent restoration efforts make seagrass meadows stand out as a significant potential carbon sink (Oresaka *et al*., 2020)

Zostera marina, commonly known as eelgrass, is a widespread species of seagrass, creating meadows in temperate and sub-tropical regions of the Northern Hemisphere (Blok *et al*., 2018). Besides their potential as carbon sinks (*e.g*., Kennedy *et al*., 2010; Dahl *et al*., 2016; Röhr *et al*., 2018), *Zostera marina* provide a range of essential ecosystem services and ecological functions. They provide habitat and food sources for a variety of marine organisms, supporting commercial and recreational fisheries (Björk *et al*., 2008). In addition, *Z. marina* help to stabilize the underlying sediment, protecting shorelines from erosion, and improving water quality (Gutiérrez *et al*., 2011), which in turn potentially contributes to the enhancement of the tourism industry and overall recreational activities.

Globally, the seagrass loss rate has been around 7% annually since the 1990s (Waycott *et al.*, 2009). More specifically, *Zostera marina* has experienced significant decline as well. For instance, regions such as the Swedish west coast, between the years of the 1980's to the 2000, the overall area covered by eelgrass had declined by 58%, and the decline was most pronounced in the inner part of the Gullmars fjord, where eelgrass beds had disappeared almost completely (Baden *et al.*, 2003; Nyqvist *et al*., 2009). Furthermore, in Chesapeake Bay on the mid-east coast of the U.S.A, the cover of *Z. marina* declined by 52% between 1993 and 2007 (Orth *et al.*, 2009). Moreover, in the United Kingdom, where *Z. marina* is a dominating seagrass species, seagrass loss has reached anywhere from 39% to 44% since the 1980s (Green *et al*., 2021).

The cause of eelgrass decline varies drastically depending on region. However, the leading causes are undoubtedly linked to anthropogenic activity. Habitat changes caused by nutrient enrichment from

sewage release and agricultural fertilization runoff can stimulate the growth of epiphytic algae on eelgrass leaves, which can shade out the seagrass and reduce its growth, and excess nutrient input, via eutrophication, increases the density and activity of phytoplankton and thus increases browning of water and light attenuation (Burkholder *et al*., 2007). Physical disturbances from human activity such as dredging (Erftemeijer & Robin Lewis, 2006), boating (Koch, 2002; Orth *et al*., 2017), anchoring (Kelly *et al*., 2019), and trawling (Guillén *et al*., 1994), can damage the eelgrass habitats directly as well as stir up sediment, which can limit light penetration and reduce eelgrass growth as well. Climate change can lead to sea level rise, which, in turn, may force eelgrass to redistribute, causing increased runoff from land (Short & Neckles, 1999). This runoff can lead to higher particle and nutrient loads and potentially result in increased albedo. Non-indigenous species introduced by climate change or maritime transport can negatively affect eelgrass by damaging the plants, eating their seeds, and competing for resources and space (Borum *et al*., 2004; Neckles 2015; Infantes *et al*., 2016). Overfishing can cause top-down cascades, decreasing the grazing of algae on eelgrass plants, leading to eelgrass decline (Moksnes *et al*., 2008; Baden *et al*., 2012).

Given the substantial decline in eelgrass meadows and the magnitude of ecosystem services they provide, restoration initiatives have been undertaken (*e.g.*, Orth *et al*., 2006; Orth *et al*., 2009). While some have been able to re-establish eelgrass through the method of replanting individual shoots, a substantial amount of shoots has also been lost during these attempts, most particularly at higher-than-expected energy events releasing the shoots from the sediment to drift away (Leschen *et al*., 2010; Eriander, 2016; Moksnes *et al*., 2016). To mitigate loss of this kind, attempts to fasten the shoots in the sediment have been made through various methods. Such as with bamboo skewers (Davis & Short 1997), PVC frames with jute-string-mesh (Leschen *et al*., 2010), rubber bands and iron rods (Phillips & MacRoy., 1990), and complex plastic 3D structures (Temmink *et al*., 2020). However, none of these structures increase the growth of morphological structures helping the shoots stay in place by themselves.

Even though many of the problems associated with the loss of eelgrass are caused by increased nutrients in the water, all living matter is dependent on nutrients as a foundational building block. *Zostera marina*, like other seagrass species, has the ability to assimilate nutrients through both

root and leaf uptake (Short & McRoy, 1984). In particular, the plant is known to take up nitrogen in the form of ammonium, nitrate, and urea, and can store excess of these nutrients in various plant tissues (Touchette & Burkholder, 2000). During the growing season (May-August; Zhang *et al*., 2016), *Z. marina* takes up nitrogen that is stored in both the leaves and rhizomes. When the growth rate decreases during the winter months, the stored nitrogen is translocated from old leaves to new leaves, where it is used for growth in the following season (McRoy & Goering, 1974; Borum *et al*., 1989). In the context of ecosystem restorations, fertilization of restored meadows can potentially increase the strength and size of the root-rhizome system at a faster pace than non-fertilized meadows, making it more resistant to high-energy events and act as a more efficient carbon sink.

To increase the growth potential during the active season, previous studies have attempted a variety of different methods for increasing the development of *Z. marina*, or other seagrass species, by controlling the speed, timing, delivery method, and dosage of nutrients supplied at seagrass meadows and in laboratory environments. Various combinations of nitrogen and phosphorus have been tested, as well as the compounds by themselves, and in different chemical forms (Bulthuis & Woelkerling, 1981; Harlin & Thorne-Miller, 1981; Dennison *et al*., 1987; Borum *et al*., 1989; Kenworthy & Fonseca, 1992; Worm *et al*., 2000; MacDonnell *et al*., 2022). In most cases, nitrogen has been shown to be the limiting factor within eelgrass meadows, and different forms of ammonium have been the most easily assimilated by the seagrass plants (Short & McRoy, 1984; Touchette & Burkholder, 2000). Thus, the addition of ammonium in the sediment has the potential to increase the eelgrass growth the most. The methods used for delivering the nutrients vary substantially between studies, especially depending on whether the experiment was conducted in the field or the laboratory. However, during studies, the fertilizer is usually placed within a container to keep it in place (Worm *et al*., 2000). Solutions such as wrapping the fertilizer in "Kleenex" tissue paper (Bulthuis & Woelkerling, 1981), plastic mesh bags (Kenworthy & Fonseca, 1992), and clay pots have been used (Harlin & Thorne-Miller, 1981). To ensure a steady and controlled supply of nutrients, most studies have utilized slow-release fertilizers. These slow-release fertilizers consist of nutrient salt compounds coated with materials like polyolefin to regulate the gradual release of nutrients, thus providing the target plant

with a continuous and consistent nutrient supply (Lawrencia *et al*., 2021).

Traditional fertilizers have several drawbacks, such as escalating the negative impacts of eutrophication (Smith & Schindler, 2009), and for slow-release nutrients specifically, the potential to release harmful plastics and microplastics into the environment (Anbumani & Kakkar, 2018). An alternative could be to use an organic fertilizer, such as the amino acid arginine. Arginine-based complexes have demonstrated significant potential as an alternative nitrogen source for terrestrial plants due to their rapid assimilation and enhancement of growth particularly that of the roots (Näsholm *et al*., 1998; Forsum *et al*., 2008; Näsholm *et al*., 2009; Gruffman *et al*., 2012; Häggström *et al*., 2021; Häggström *et al*., 2023). The increase in root growth could be particularly important during restoration efforts of *Zostera marina* to withstand stronger-than-expected currents. Other especially promising qualities found for arginine-based nitrogen sources are: (1) no need for plastic coating, (2) significantly smaller amounts are needed, compared to traditional fertilizers, and (3) on land the arginine has a quality that makes it stick to the soil and not flow away to the nearest stream during rainfall (Inselsbacher *et al*., 2011). This could translate to the marine environment such as that the nutrients stay in the sediment instead of being flushed away during porewater exchange.

Research question and objectives

The questions this thesis aims to answer are: (1) whether the growth of *Z. marina* in mesocosms differs when enriching the sediment with an organic fertilizer (arginine), and (2) if there is a difference when treating the sediment with a traditional inorganic slow-release fertilizer.

The hypothesis is that several morphological parameters related to the growth of *Z. marina* will be larger when treated with organic fertilizer compared to inorganic fertilizer and control.

If so, the potential to use organic fertilizers during restoration efforts is worth further research and consideration.

Methods

Mesocosm setup

Fifteen aquaria (60 x 38 x 36 cm) were positioned in a greenhouse, exposed to natural sunlight, at the Kristineberg Center for Marine Research and Innovation in Fiskebäckskil on the Swedish west coast. These aquaria served as the experimental

environment of the study. The experiment ran from the 30th of May to the 4th of July.

Sediment for the aquaria was collected adjacent to the *Zostera marina* meadow where the sampling of shoots was conducted (Kristineberg Bay; Lat 58.249272, Long 11.446991). The area for sediment collection did not extend beyond 10 m from the meadow's edge, and the sampling depth was limited to 10 cm into the sediment. The collected sediment was thoroughly mixed in large buckets using a shovel. The sediment was then evenly distributed among all the aquaria, with each aquarium containing sediment that reached a depth of 10±1 cm. The sediment was left to settle for one week before starting the experiment.

A continuous water flow of 1±0.3 liters per minute of unfiltered surface seawater (from a depth of 7 m) was maintained for each aquarium. This flow rate was assessed and adjusted thrice weekly to ensure consistency.

Zostera marina collection and processing

Zostera marina was collected along the shallow meadow border. The collected shoots were promptly processed; individual shoots were tagged with a labelled zip-tie. Measurements of the belowground (roots length and count) and aboveground (leaves area and count) parts of seagrass were done through a comprehensive image of each *Z. marina* sample, captured using a Sony A6000 camera with a Sony E18-55mm F3.5-5.6 OSS lens. All image analyses were performed using ImageJ (version 1.53t) line and polygon tool. Additional measurements, such as wet weight was collected by shaking the entire shoot for 15 seconds before measuring on a Mettler Toledo B3002 DeltaRange scale. As well as the volume displaced by the shoot belowground tissue was recorded by submerging the root-rhizome in a measuring vial containing surface seawater until the water level aligned with the highest root section.

Experimental Design

In order to assess the potential effects of different fertilizers on *Z. marina* growth parameters, one organic amino-acid fertilizer, one inorganic fertilizer, and control were used. The nutrient sources (treatments), arGrow® (L-Arginine within clay complex) granules, and Substral Osmacote 16:2:14 were measured for 15 mg Nitrogen content (as determined during a pilot study) on a Mettler Toledo B502 scale. The nutrients were placed within a fine plastic mesh bag (2x5cm) and sealed with a PimeMatik impulse sealer.

Picture 2: Photograph of the experimental set-up with the 15 aquaria that were used as mesocosms.

During the evening of the same day as the *Z. marina* collection, six samples were randomly placed in each of the 15 aquaria (Picture 1-2, after first sampling time turned 11 aquaria) with the help of R Studio's sample function. A vertical hole approximately 7 cm deep was dug with a finger, and a treatment bag was positioned at the base of the hole. The *Z. marina* shoot was carefully placed atop the bag, and the hole was refilled with the surrounding surface sediment. Sample placement followed Eriander's (2016) spacing recommendations of 16 shoots m-2 (which equates to at least 25 cm radius to another shoot) to prevent nutrient competition.

After a week and once every subsequent six weeks, a single *Z. marina* sample was collected from each aquarium. Using a garden shovel ~10 cm away from the shoot's base, the sediment and shoot sample were carefully lifted. The sediment was gently shaken off within the water to refill the hole. The treatment bag was discarded, and the sample was immediately transported to the laboratory for analysis. After rinsing the shoots with surface water, the previously described measurements; root length and count, leaf area and count, wet weight, and root volume, were repeated for each collected sample.

Statistical analyses

Using Excel Version 2307 (Build 16.0.16626.20.170) linear regression analyses were used to assess the correlative relations for each treatment (organic fertilizer, inorganic fertilizer, and Control) between measured changes in shoot variables (total and average root length and root count, leaf area and count, wet weight, and root volume) over time (T1-T6). The change of a variable was defined as the difference between the measurement at the time of sampling and the measurement prior to the start of the experiment.

Additionally, a Pearson correlation matrix was produced to assess the relationship between the measured variables. Using the R function "cor", all measured variables were pairwise compared and representive correlation coefficient values between each

variable were produced. For this, R version 4.1.1 was used, as well as for the following analyses.

To determine the differences between treatments at each sampling time, for each of the measured variables, either a one-way ANOVA or a Kruskal-Wallis test was performed based on the results of a Shapiro-Wilks homogeneity test. Following significant results of Kruskal-Wallis tests, the DunnTest was employed within the R package "dunn.test" Version 1.3.5. While after significant results following the one-way ANOVA tests, the post-hoc test TukeyHSD was used.

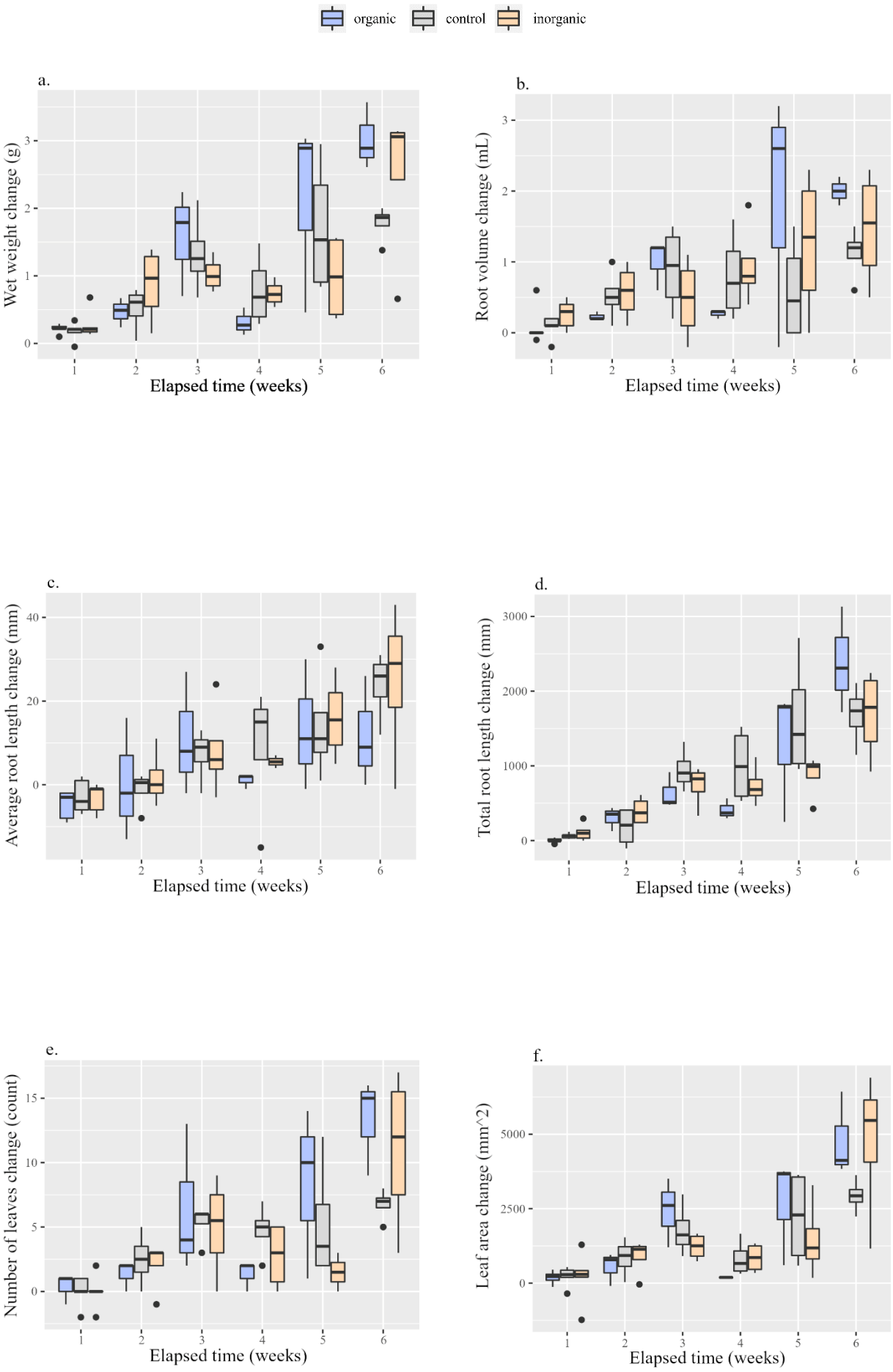
Furthermore, to examine the rate of shoot growth over time, calculations of the growth rate by dividing the change of all measurements by the number of days under treatment was made for each respective sampling time. For each of the treatments, assessment of the homogeneity using the Shapiro-Wilks homogeneity test was performed prior to either a one-way ANOVA or a Kruskal-Wallis test, based on homogeneity results. Following with either a DunnTest for Kruskal-Wallis, or TukeyHSD test for ANOVA, after significant results. Based on results of the pairwise comparison between the timesteps, a hexagonal visualisation over the significant p-values between each timestep was created.

Results

Growth analysis

Multiple one-way ANOVAs and Kruskal-Wallis tests were used to test the hypothesis whether the growth of *Zostera marina* in mesocosm would differ when grown with the added organic nitrogen source arginine. Figure 1 shows that few of the measured morphological variables differed in the change that occurred since first sampling time and the respective elapsed time. These include wet weight, root volume, average root length, total root length, number of leaves, and leaf area (*Figure 1a-f*). Significant differences were found at week 6, where the number of roots change were found to have increased more than that of the control and inorganic fertilizer ($p < 0.05$; *Figure 1g*). The number of roots at week 6 changed with that of an additional average count of 41 for arginine, while 23.5 and 26.5 for control and inorganic fertilizer, respectively.

There appears to be no set pattern to the measurements between each sampling time for each variable (*Figure 1a-g*), except when comparing the variables at sampling time 6 across variables, where there appears to be a commonality of organic fertilizer and inorganic fertilizer to be larger than the control.


organic
control
inorganic
a.
Wet weight change (g)
Elapsed time (weeks)
b.
Root volume change (mL)
Elapsed time (weeks)
c.
Average root length change (mm)
Elapsed time (weeks)
d.
Total root length change (mm)
Elapsed time (weeks)
e.
Number of leaves change (count)
Elapsed time (weeks)
f.
Leaf area change (mm^2)
Elapsed time (weeks)

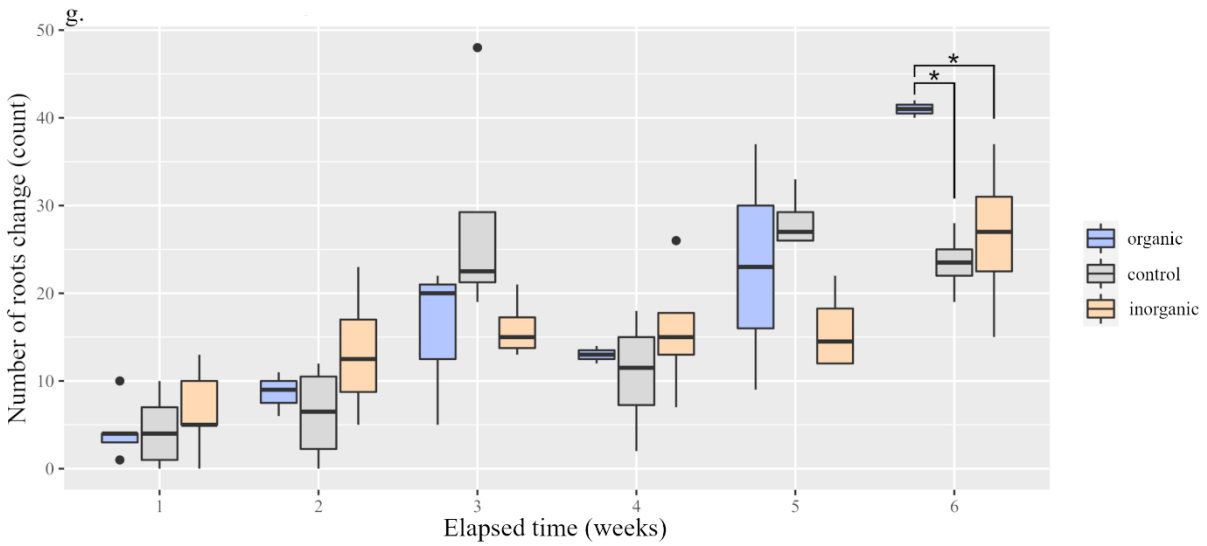
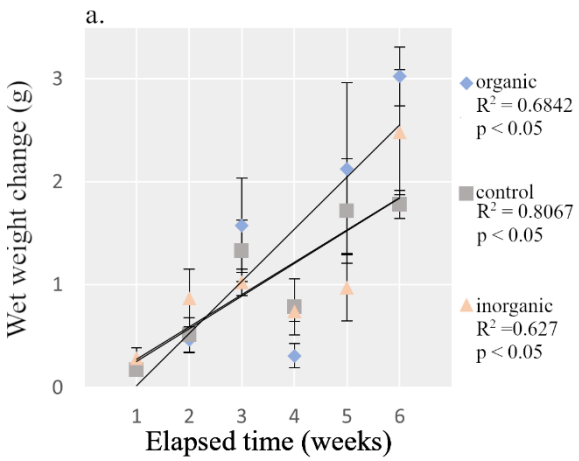


*Figure 1: The change in each measured variable, a) wet weight (grams), b) root volume (milliliter), c) average root length (millimeter), d) total root length (millimeter), e) number of leaves (count), f) leaf area (millimeter^2), and g) number of roots (count). Change is defined as the difference between the measurement made prior to the experiment start to the measurement made after sampling, also called growth. Each sample is unique for every sampling time. Treatments are visualized as blue for organic fertilizer (arginine), grey for control, and orange for inorganic fertilizer. The sample size is 5 for each treatment for week 1, for each of the following sampling times (week 2-6) organic fertilizer has a sample size of 3 while control and inorganic fertilizer has a sample size of 4. The box plots represent the 25th to 75th quartile, visualize the median as a line and whiskers show the spread. Outliers are shown as dots. Significant differences are visualized with * when p < 0.05.*

Time analysis

A regression analysis was performed for each measured variable for each of the different treatments. This was done to investigate whether there was a general trend over time for the change in the measured variables, and if there was a difference between treatments. Figure 2 illustrates the results of these analyses, revealing statistical trends over time for nearly all treatments, for most measured variables (p-value < 0.1).

There were two results which did not show any statistical trends, these include the number of roots for the control treatment (p = 0.12) and the number of leaves for the inorganic fertilizer treatment (p = 0.12).



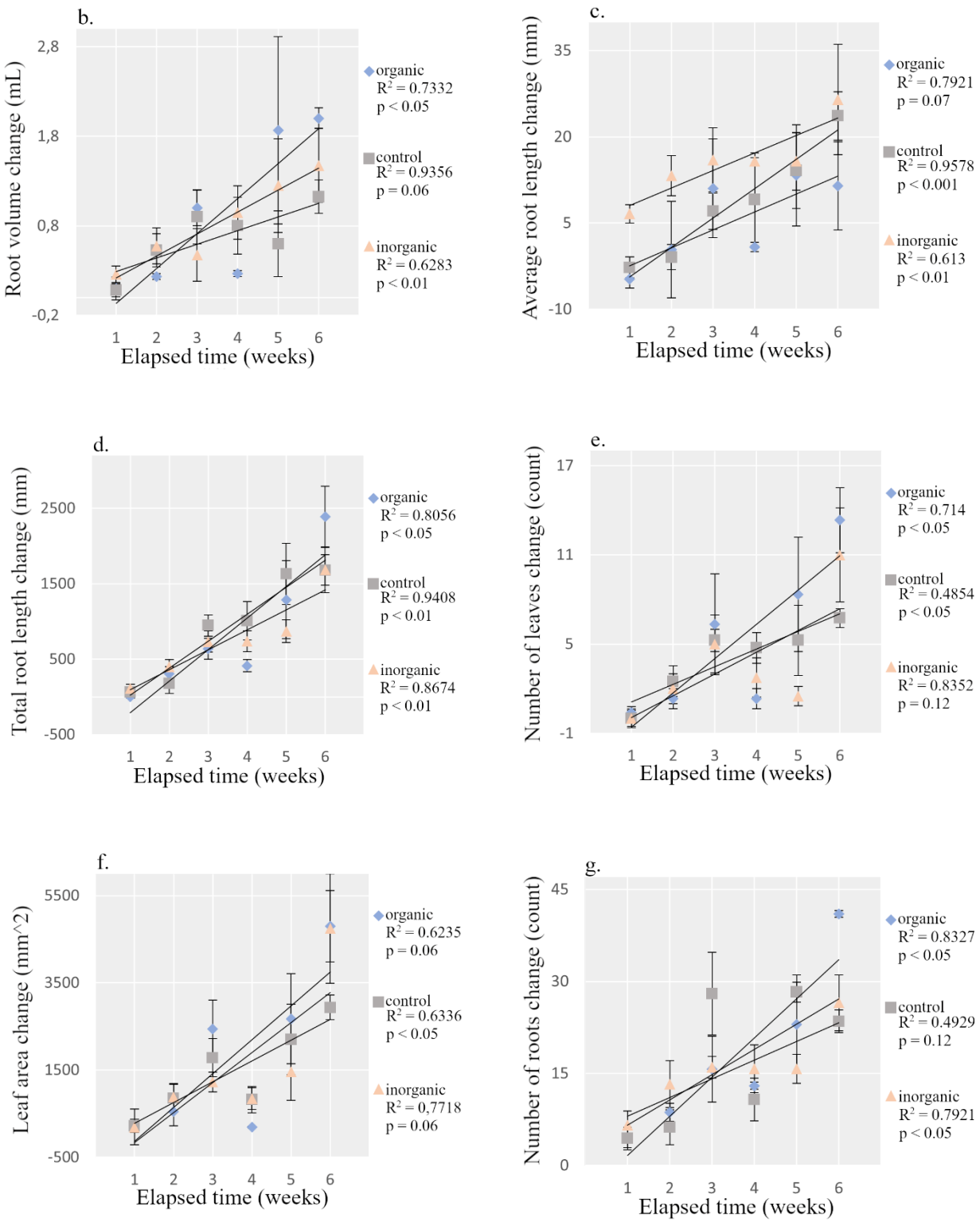
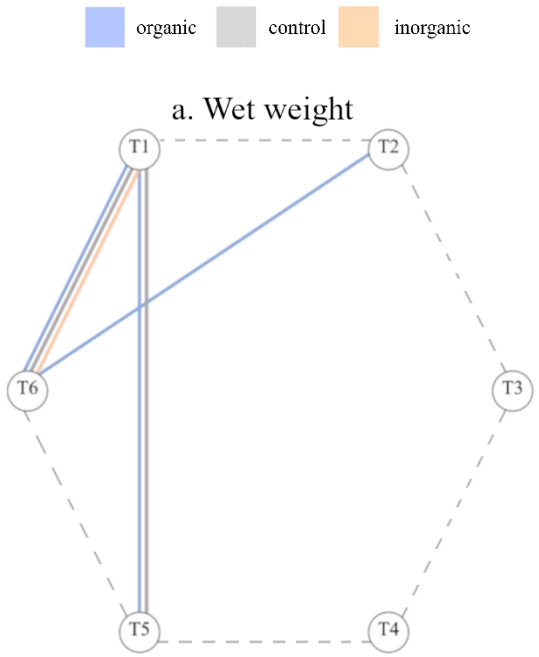


Figure 2: The change in each measured variable, a) wet weight (grams), b) root volume (milliliter), c) average root length (millimeter), d) total root length (millimeter), e) number of leaves (count), f) leaf area (millimeter^2), and g) number of roots (count). Change is defined as the difference between the measurement made prior to the experiment start to the measurement made after sampling, also called growth. Each sample is unique for every sampling time. Treatments are visualized as blue diamonds for organic fertilizer (arginine), grey squares for control, and orange triangles for inorganic fertilizer. The sample size is 5 for each treatment for time one, for each of the following sampling times organic fertilizer has a sample size of 3 while control and inorganic fertilizer have a sample size of 4. The mean is visualized as respective shapes and whiskers show the standard error. The R2 and p-value are shown on the side of each graph.

Growth rate analysis

Multiple one-way anovas and Kruskal-Wallis tests were performed to test whether the growth rate of *Zostera marina* differed between the sampling times and if this varied between treatments. Figure 3 depicts the result of these analyses by the use of lines indicating a significance ($p < 0.05$). Significant results were revealed for each measured variable between sampling week 1 and 6, with the exception being the measured variable total root length change, which does not show any significant differences in the inorganic fertilizer treatment between each of the sampling times (*Figure 3d*). Generally sampling week 6 and 5 show significant differences to sampling week 1 in most variables and treatments, this is also true between sampling time 6 and 2 but less so. Deviations include leaf area where fertilizer treatment is significantly different to sampling week 1 already from sampling time 3 and upward (*Figure 3f*).



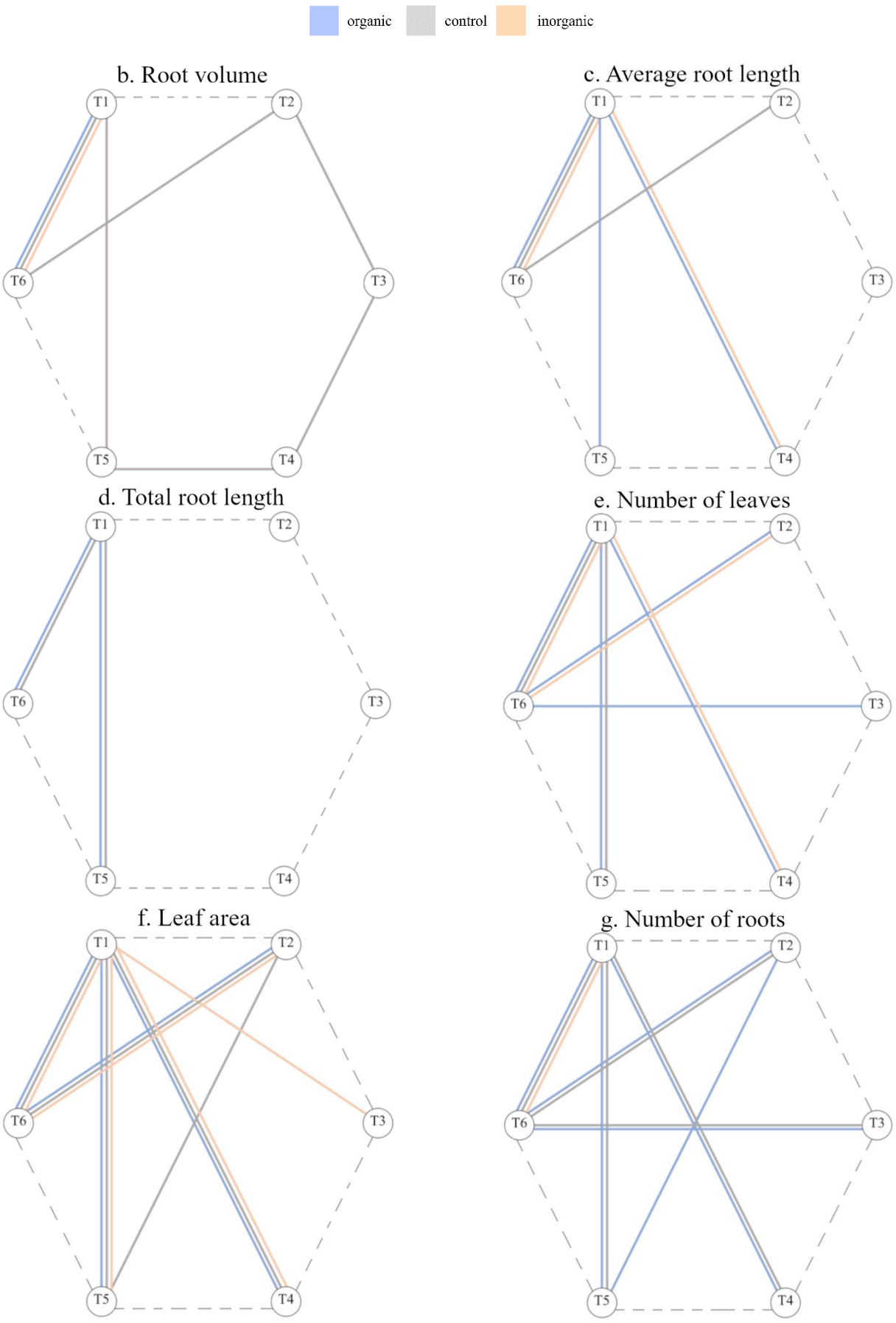


Figure 3: Visualization of pairwise comparison between sampling times T1-T6, i.e., sampling week 1-6. A significant value between sampling times ($p < 0.05$) are illustrated with a line connecting the two sampling times. (specific p-values can be found in Appendix A1-3). The color of the line indicates treatment: blue represents organic fertilizer (arginine), grey control and orange inorganic fertilizer. Each measured variable is visualized with its own graph: a) wet weight (grams), b) root volume (milliliter), c) average root length (millimeter), d) total root length (millimeter), e) number of leaves (count), f) leaf area (millimeter^2), and g) number of roots (count).

Variable correlation analysis

A correlation matrix was produced to test how the change in our measured morphological variables were related to each other, regardless of treatment. Figure 4 visualizes these results and indicates that all the included variables are positively correlated to some degrees. The most correlated measurement were leaf area change to that of wet weight change, and close thereafter number of leaves change to that of wet weight change. The average root length change and the root volume change were the two measured variables with the lowest correlation with all the other measured variables.

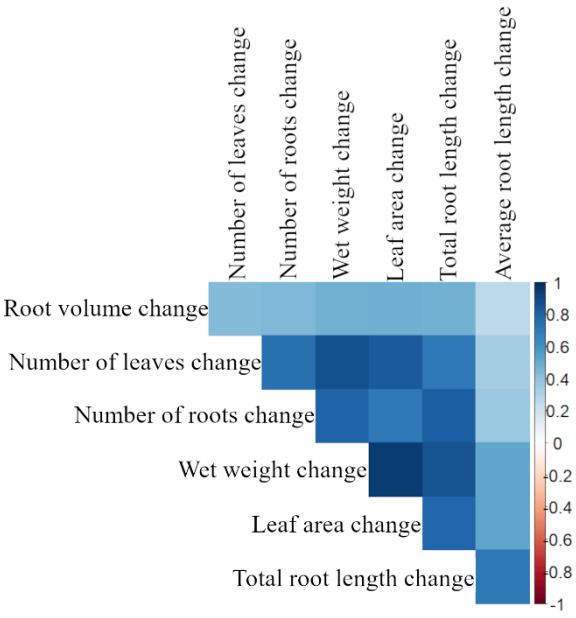


Figure 4: The correlation plot illustrates the relationship between each of the measured variables using the Pearson correlation method. The measured variables include: wet weight change (grams), root volume change (milliliter), average root length change (millimeter), total root length change (millimeter), number of leaves change (count), leaf area change (millimeter^2), and number of roots change (count). Change is defined as the difference between the measurement made prior to the experiment start to the measurement made after sampling, also called growth. The correlation between the variables is represented with a color range of blue to red. Where blue indicates a positive correlation and red a negative one. The darker the color the stronger the correlation.

Discussion

In this thesis, we set out to investigate whether or not there would be a significant difference between the growth of *Zostera marina* in mesocosms when enriched with either an organic fertilizer (arginine) or a inorganic nitrogen-heavy fertilizer, as well as compare this with controls. What we found was that the number of roots for the organic fertilizer treatment was significantly higher compared to both inorganic fertilizer and controls at the last sampling time, week 6 (*Figure 1g*). The same trend could also be seen for the total length of roots but was not significant, presumably due to the low amount of replicates available (*Figure 1d*). These findings support the main hypothesis that the organic fertilizer, arginine, can be a more effective nitrogen source than inorganic fertilizers for marine vascular plants such as *Z. marina* and be a stimulant for increased root growth. This is in line with previous findings for terrestrial vascular plants where organic nitrogen sources have been found to be easily assimilated and increase the growth of plants (*e.g.*, birch and pine) and especially increase root growth. (Näsholm *et al.*, 1998; Gruffman *et al.*, 2012; Häggström *et al.*, 2021; Häggström *et al*., 2023).

The contradiction to other studies arises when looking into traditional inorganic fertilizers. In previous eelgrass studies, ammonium has been found to be the most easily assimilated inorganic nitrogen source (Short & McRoy, 1984; Touchette & Burkholder, 2000). In the fertilizer we used, ammonium was the main component, and in our results, we found no significant difference between the traditional fertilizer to the controls when looking at all the different measured variables between all the different sampling times (*Figure 1a-g*). Two possible explanations for this are that the nutrient needs are already being met by either the sediment or the water inflow. However, we were not able to conduct any nutrient analysis of the sediment; the sediment we used was muddy organic sediment, which according to Touchette & Burkholder (2000) is generally regarded as nitrogen limited, the same is also generalized for sandy sediments. The primary production during the months of June and July are relatively high in the Gullmars fjord (Lindahl *et al.*, 2009), possibly indicating a high availability of nutrients in the water column. In such a case, this could explain the lack of significant differences between the inorganic fertilizer treatment and control. This could be further argued when one takes into account that *Zostera marina* has been found to sometimes uptake 90% or

more of its nitrogen from leaf assimilation, however, it is important to note that this number can vary drastically (from 30 - 90%) depending on habitat (Touchette & Burkholder 2000).

Additionally, it is important to consider the type of nutrient selected, and if its slow-release properties are suitable for the marine environment, as it is intended for terrestrial use. Considering that other studies have used the same type coating on their nutrients (Osmocote), and have found an effect (Kenworthy & Fonseca, 1992; Tanner & Parham 2010; MacDonnell *et al*., 2022), it is reasonable to assume that we as well should have recorded a difference if no other variables differed. However, an additional aspect which could heavily affect the results are the amount of nutrients used. Due to the fact that comparing our dosages to other studies proved to be challenging, because of the differences in delivery methods, we opted to maintain consistency in nitrogen dosage between the organic fertilizer and the inorganic fertilizer. This decision was informed by a pilot study we conducted that revealed a noticeable effect when using organic nitrogen at the 15 mg-N dosage. Given that the properties of the organic fertilizer, arginine, suggests that a smaller dose is needed compared to inorganic fertilizers, it is conceivable that the amount of inorganic fertilizer we used may have been insufficient.

Still, the reason for requiring a lower amount of arginine to achieve an effect may be attributed to its unique properties of adhering to the soil or sediment, preventing it from being flushed away. In this context, it is essential to consider the hydro dynamics of the aquaria. Given the relatively low flowrate available in relation to the aquaria size, the resulting currents created are slow and of low energy. Furthermore, with the water outlet situated on the opposite side of the aquaria at the surface level, there is limited flow across the sediment-water diffusion barrier. Consequently, there is supposedly minimal exchange in porewater, and the aquaria act as sinks. As a result, the inorganic nutrients should have been able to remain in place and been available to be assimilated by the shoots.

Building upon the assumption that the shoots may already be nutrient-saturated from the water column, this leaves us with the question: why do we observe a disparity in the root growth between the arginine and fertilizer treatments? We hypothesize that this distinction may be attributed to the organic nutrient uptake mechanism inherent to the type of nutrient we use. Most plants have roots that are colonized by mycorrhiza which aid in the

uptake and usage of organic nutrients (Smith & Read 2010). However, in eelgrass and other seagrass species for that matter, little research has been done to explore the topic of mycorrhiza. Nevertheless, a study published by Nielsen *et al*., (1999) found no vesicular-arbuscular mycorrhiza within *Zostera marina* nor *Thalassia testudinum*. This means that for seagrass to utilize organic nutrients there needs to be a different pathway. One active mechanism mentioned by Näsholm *et al*., (2009), when they looked at terrestrial plants, is the lysine histidine transporter (LHT1) which is highly present in emerging roots. Thus, there is reason to suspect a potential reason for our findings of a higher number of roots being the beneficial effect of having newer/more roots to access the organic nutrient supplies. Questions still arise as to why it would be beneficial to increase the number of roots to access the organic nutrients when nutrient needs are possibly satisfied, as discussed before, through the water column. One possible explanation is that organic nutrients are more readily assimilated, potentially prompting the shoots to exhibit a greater inclination to assimilate more from the arginine source.

Intuitively we wanted to investigate whether the shoots were growing at all over time, if the measurements we were taking were completely stochastic or if the growth was as expected, increasing over time. We performed regression analysis for each treatment against sampling time, for every measured variable, and most of the results indicated statistical trends (p-value < 0.1; *Figure 2*). The two results that did not show statistical trends in the regression analyses include the number of leaves for the inorganic fertilizer treatment, and the number of roots for the control, with p-values values rounded to 0.12.

The non-linear regression observed in the number of leaves can be attributed to the inherent characteristics of plants. As documented by various authors (*e.g.*, Pedersen & Borum, 1993), seagrass undergoes a natural process of leaf shedding. Consequently, it is logical to infer that the number of leaves cannot perpetually increase since the last sampling time since not all leaves are shed and grown equally. However, we did see a significant trend for the other treatments for the number of leaves. So perhaps the basis of this stochasticity is not the treatment but caused by the fault of few replicates and random selection of which plant goes into which treatment, and that it just so happened to be the fertilizer treatment that received the plants with the older leaves, that were closer to their shedding stage.

The second non-significant p-value reported in Figure 2 is the number of roots for the control treatment. The lack of significance appears to be mainly driven by an unusually elevated number of roots recorded at the third sampling time (*Figure 2g*). Notably this elevated count also stands out when compared to other treatments and across different sampling times. The underlying reasons for this outlier remain unexplained, with potential factors such as methodological errors or inherent randomness within the sampled data warranting consideration.

We further investigated the growth of the subjects by calculating the rate of growth and performing analyses to inquire into whether there are differences among the sampling occasions. What we discovered was that during our experimental period, the growth rate would be significantly different, for most measured variables and treatments, when looking at earlier sampling points to the later (*Figure 3a-g*). More specifically the growth rate was generally different between the first sampling week (T1) and the last (T6). But also noteworthy for many measurements was that the second half of the experiment (T4-6) was different from the first (T1-2). There are few studies that measure the growth of eelgrass (or any seagrass for that matter) in mesocosms and I have not found any reported growth rates, especially over such a short time span. However, some studies that have performed mesocosm studies for seagrasses have opted to use an acclimatization period before initiating their experiment (*e.g.,* Haynes *et al.,* 2000; Bernardeau-Esteller *et al.,* 2015; Kumar *et al.,* 2017; Koch *et al*., 2022). Although this is worth consideration for studies such as this, we do believe that with the method used for this study, an acclimatization period for the number of aquaria we used might have increased the number of uncontrolled factors, and been unpractical considering our method of planting the eelgrass shoots with the nutrients. Another important factor to consider is the difference in light and temperature between the sampling times which could have been drastically different and impacted the speed of growth. However, a study by Dennison and Alberte (1982) found that shallow eelgrass meadows, such as those we sampled from, experienced no significant reduction in growth when artificially shaded for a period of 1-2 weeks. Nevertheless, in hindsight, a measurement of the irradiation intensity for the whole duration of the experiment would have proven interesting, and worth implementing if the experiment is to be reproduced.

Regarding temperature, Eriander (2017) found that during a mesocosm temperature manipulation experiment, eelgrass shoots grow larger leaves at higher temperatures (20^{o}C) compared to when in lower temperatures (12^{o}C). If we make an assumption and generalize that growth is one single factor, we would expect to notice a significant difference in our experiment depending on time caused by a failure in the surface water (7m depth) inflow, which at the last two weeks of the experiment failed and caused us to switch to a deepwater source (30m depth) considerably colder (mean difference of ~7^{o}C).

To assess whether an assumption such as the previous paragraph made can be applicable to our data we performed a correlation analysis, to see how much the growth of one measured variable related to another (*Figure 4*). As anticipated from Figure 3, all measurements are increasing over time, and thus the correlation plot would be positive for all measured variables. It is essential to emphasize these relationships are not universally generalizable but are specific to the conditions of our experiment. Notably, certain measurements exhibited stronger correlations than others, such as the relationship between the wet weight to the leaf area as well as to the number of leaves. Indicating that the leaves were more prominent in promoting the increase in biomass of the shoots compared to the root growth. Nevertheless, the contribution of root growth to overall biomass was not far thereafter and should not be ignored.

Putting our results in the context of the potential of arginine to be used within restoration efforts is difficult at the current state with more research being needed before drawing any hard conclusions. With that being said, there is an increase in the number of roots having the potential to increase the force needed to uproot the shoots, increasing their rate of survival at replantation sites. However, Moksnes *et al.,* (2016) argue that additional anchoring methods, such as adding bamboo skewers to fasten the shoot in the sediment, are unnecessary in certain conditions and that transplanted shoots reach a strength equal to that of naturally occurring meadows after a period of 10 days. Hence, they recommend not using anchoring methods, and instead plan the replantation event with a following calm week in mind. Taking this into consideration, there was only a noticeable difference in the number of roots grown in arginine fertilized medium after a period of 6 weeks, 42 days (*Figure 1g*). Meaning that the strength of the roots would have been strong enough to withstand the environmental conditions

long before an effect of the arginine could be detected.

Placing our findings within the broader context of climate change, the increase in number of roots has the potential to increase the sequestration of carbon with the help of increased belowground growth as well as the sediment stabilizing properties. However, growth of the measured variables which could potentially increase carbon sequestration more, were not significant. For instance, an increase in wet weight has the potential to boost autochthonous carbon sequestration, the increase in leaf area can aid in capturing allochthonous carbon for subsequent sequestration, and an increase in total root length can potentially increase stability more than the number of roots and dead roots present a source of carbon sequestration.

The major concern that could be presented regarding the fertilization of eelgrass meadows, regardless of if arginine is used or not, is the increase of eutrophication, which is a major problem within the Swedish marine environment. Even if arginine is potentially safer to use, as it adheres to the sediment, and is more completely assimilated, it could further down the line be released in different forms. However, the total amount of nitrogen used within this study with 90 different shoots equates to 1350 mg N. That is roughly ⅓ of the nitrogen one person releases upon a single urination in the ocean (Estimated via measurements by Pradhan *et al*., 2007 and assuming average urination happens 4 times per day).

In conclusion, our study suggests that organic fertilizers such as arginine can serve as a more effective nitrogen source compared to inorganic fertilizers for *Zostera marina*, in stimulating growth of new roots. However, the effectiveness of the inorganic fertilizers was not evident in our results, which is contrary to other studies, possibly due to factors such as nutrient dosage and low sample size. Our findings emphasize the complexity of nutrient dynamics in marine ecosystems and broaden the ground for research on organic nutrient uptake in seagrasses. This research has implications for seagrass restoration and carbon sequestration in the face of environmental change.

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Appendix

Appendix A1: Pairwise comparison between all timesteps and measured variables for the organic fertilizer (Arginine) treatment. All tests are the non-parametric Kruskal-Wallis.

	Wet weight	Root volume	Avg. root length	Tot. root length	Nr. of leaves	Leaf area	Nr. of roots
T1-T2	.568	.350	.381	.460	.394	.189	.890
T1-T3	.266	.081	.099	.312	.394	.076	.490
T1-T4	.234	.058	.042	.068	.039	.017	.062
T1-T5	.017	.025	.017	.043	.029	.011	.053
T1-T6	.003	< .001	.012	.030	.002	< .001	.004
T2-T3	.629	.468	.488	.808	1	.679	.527
T2-T4	.581	.388	.298	.330	.279	.334	.052
T2-T5	.105	.240	.176	.251	.235	.269	.043
T2-T6	.035	.021	.145	.198	.043	.045	.002
T3-T4	.945	.890	.729	.465	.279	.581	.241
T3-T5	.255	.654	.510	.366	.235	.490	.214
T3-T6	.105	.112	.446	.297	.043	.112	.027
T4-T5	.284	.756	.755	.862	.916	.890	.945
T4-T6	.120	.147	.677	.754	.345	.300	.301
T5-T6	.629	.255	.917	.889	.402	.369	.334

Appendix A2: Pairwise comparison between all sampling times and measured variables for the Control treatment. Wet weight, total root length, and number of roots were performed using a Kruskal-Wallis non-parametric. Root volume, average root length, number of leaves, and leaf area p-values were produced with a one-way anova

	Wet weight	Root volume	Avg. root length	Tot. root length	Nr. of leaves	Leaf area	Nr. of roots
T1-T2	.346	.999	1	.481	.639	.999	.257
T1-T3	.099	< .001	.649	.338	.041	.070	.217
T1-T4	.080	.751	.489	.164	.077	.046	.013
T1-T5	.035	< .001	.155	.025	.041	< .001	.008
T1-T6	.004	.007	.008	.009	.006	< .001	< .001
T2-T3	.501	.004	.817	.809	.598	.186	.923
T2-T4	.442	.939	.678	.514	.770	.131	.203
T2-T5	.269	.003	.280	.147	.598	.002	.149
T2-T6	.061	.025	.019	.070	.174	.001	.033
T3-T4	.923	.025	1	.681	1	1	.239
T3-T5	.665	1	.920	.226	1	.288	.179
T3-T6	.230	.939	.210	.116	.946	.220	.041
T4-T5	.737	.022	.977	.425	1	.384	.866
T4-T6	.269	.149	.312	.245	.843	.300	.387
T5-T6	.442	.924	.720	.717	.946	1	.486

Appendix A3: Pairwise comparison between all sampling times and measured variables for the inorganic fertilizer treatment. Wet weight, average root length, total root length, number of leaves, leaf area, and number of roots were performed using a Kruskal-Wallis non-parametric test. Root volume p-values were produced with a one-way anova.

	Wet weight	Root volume	Avg. root length	Tot. root length	Nr. of leaves	Leaf area	Nr. of roots
T1-T2	.181	.662	.361	.791	.349	.302	.346
T1-T3	.165	.312	.075	.501	.223	.028	.297
T1-T4	.130	.339	.071	.091	.115	.022	.148
T1-T5	.081	.339	.006	.081	.025	.005	.089
T1-T6	.003	.003	.003	.081	.001	< .001	.003
T2-T3	.962	.990	.413	.699	.789	.269	.923
T2-T4	.866	.994	.399	.176	.544	.230	.631
T2-T5	.701	.994	.083	.161	.215	.093	.471
T2-T6	.113	.091	.057	.161	.029	.008	.055
T3-T4	.904	1	.981	.334	.734	.923	.701
T3-T5	.737	1	.360	.310	.331	.564	.532
T3-T6	.125	.257	.278	.310	.055	.124	.068
T4-T5	.829	1	.373	.961	.528	.631	.810
T4-T6	.156	.236	.289	.961	.114	.150	.150
T5-T6	.230	.236	.866	1	.343	.337	.230