



universität
wien

MASTERARBEIT / MASTER'S THESIS

Titel der Masterarbeit / Title of the Master's Thesis

„Colonization of plants and mycorrhizal fungi on newly
arisen small islands in Denmark“

verfasst von / submitted by

Patricia Pérez Rodríguez

angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of
Master of Science (MSc)

Wien, 2018 / Vienna 2018

Studienkennzahl lt. Studienblatt /
degree programme code as it appears on
the student record sheet:

A 066 833

Studienrichtung lt. Studienblatt /
degree programme as it appears on
the student record sheet:

Ecology and Ecosystems

Betreut von / Supervisor:

ao. Univ.-Prof. Mag. Dr. Irmgard Greilhuber

Colonization of plants and mycorrhizal fungi on newly arisen small islands in Denmark



Patricia Pérez Rodríguez

Master Thesis

University of Vienna

October 2018

Supervisor: Irmgard Krisai-Greilhuber

Colonization of plants and mycorrhizal fungi on newly arisen small islands in Denmark

Patricia Pérez Rodríguez

ABSTRACT

Primary succession is a main topic of the ecological research as it is a key to understanding community assembly. However, most studies focused on plants species turnover, while the role of microorganisms remained practically unknown.

In this study I analyzed the biotic feedback on plant growth from soils of different successional age. The soils come from a set of sites on a sedimentation coast in Denmark and on the small islands near the coast. In order to separate the effects of beneficial microorganisms (mycorrhizas) and antagonistic microorganisms (soil-borne pathogens), I studied both mycorrhizal and non-mycorrhizal plant species.

My results show that plant growth performance is affected by soil age. I found a tendency of plant species to have a higher growth rate in soils from newly established islands – probably with lower amounts of both pathogenic and mycorrhizal fungi - compared to older ecosystems - where both detrimental and beneficial microorganisms have presumably accumulated over time-. This observation becomes more evident when looking at grasses (mycorrhizal plant species) separately. I also found that the symbiotic strategy of plants seems to influence their performance, depending on successional age of soil.

These findings contribute to a better understanding of the tripartite relation plant-pathogen-arbuscular mycorrhizal fungus and its role in primary succession.

Key-words: Arbuscular mycorrhizal, Primary succession, Sedimentary islands, Soil microorganisms, Pathogens, Plant community.

Colonization of plants and mycorrhizal fungi on newly arisen small islands in Denmark

Patricia Pérez Rodríguez

ZUSAMMENFASSUNG

Primäre Sukzession ist ein Hauptthema der ökologischen Forschung als Schlüssel zum Verständnis der „Community Assembly“. Trotzdem konzentrierten sich die meisten Studien auf die Sukzession der Pflanzenarten, während die Rolle der Mikroorganismen praktisch unbekannt bleibt.

In der vorliegenden Studie analysierte ich die biotische Rückmeldung des Pflanzenwachstums auf Böden unterschiedlichen Alters. Die Böden stammen von einer Reihe von Standorten an einer Sedimentationsküste in Dänemark und von den kleinen Inseln in der Nähe der Küste. Um die Effekte von nützlichen (Mykorrhiza) und antagonistischen Mikroorganismen (bodenbürtige Krankheitserreger) zu trennen, untersuchte ich sowohl Mykorrhiza- als auch Nicht-Mykorrhiza-Pflanzenarten.

Meine Ergebnisse zeigen, dass die Pflanzenwuchsleistung vom Bodenalter beeinflusst wird. Ich habe eine Tendenz der Pflanzenarten gefunden, eine höhere Wachstumsrate in Böden von neu errichteten Inseln zu haben – wahrscheinlich mit geringeren Mengen von sowohl pathogenen als auch mykorrhizierten Pilzen – im Vergleich zu alten Ökosystemen – wo sich sowohl schädliche als auch nützliche Mikroorganismen im Laufe der Zeit angesammelt haben-. Diese Beobachtung wird deutlicher, wenn man Gräser (Mykorrhiza-Pflanzenarten) separat betrachtet. Ich fand auch, dass die symbiotische Strategie der Pflanzen ihre Leistung zu beeinflussen scheint, abhängig vom Alter des Bodens.

Diese Ergebnisse tragen zum besseren Verständnis der dreigliedrigen Beziehung Pflanze-Pathogen-arbuskuläre Mykorrhizapilzes und ihrer Rolle in der Primärsukzession bei.

Key-words: Arbuskuläre Mykorrhiza, Primäre Sukzession, Sedimentierte Inseln, Bodenmikroorganismen, Krankheitserreger, Pflanzengemeinschaft.

INDEX

1. <u>ACKNOWLEDGEMENTS</u>	2
2. <u>INTRODUCTION</u>	2
3. <u>AIMS</u>	3
4. <u>ARBUSCULAR MYCORRHIZA AND SOIL-BORNE PATHOGENS</u>	3
5. <u>MATERIAL AND METHODS</u>	5
5.1. STUDY AREA	5
5.2. SAMPLING DESIGN	5
5.3. EXPERIMENT SET UP	6
5.4. PLANT GROWTH RATE AND MYCORRHIZAL COLONIZATION MEASUREMENTS	6
5.5. STATISTICS	6
6. <u>RESULTS</u>	7
7. <u>DISCUSSION</u>	9
8. <u>CONCLUSIONS</u>	14
9. <u>FUTURE RESEARCH PERSPECTIVES</u>	15
10. <u>REFERENCES</u>	15
11. <u>SUPPLEMENTARY MATERIAL</u>	22

1. ACKNOWLEDGEMENTS

I want to especially thank Hans Henrik Bruun (Ecology and Evolution Department, University of Copenhagen) and Irmgard Krisai-Greilhuber (Department of Botany and Biodiversity Research, University of Vienna). I also thank the collaboration of all the work team members at the University of Copenhagen: Álvaro López-García, Søren Rosendahl, Rasmus Kjøller, Marta Gil Martínez and Camila Maciel Rabelo Pereira.

2. INTRODUCTION

Since its origin, community ecology research has been concerned about primary succession as a main issue. This process of ecosystem development when plants, animals and microbes colonize barren surfaces is a challenging subject as it is hard to find unspoiled study systems that can be representative of this phenomenon. Nevertheless, its understanding is a fundamental tool to better examine ecosystem features such as fertility, productivity and diversity, as well as to improve the ability to mitigate unfavorable circumstances (Walker & Del Moral, 2003; Jing *et al.*, 2015).

The interaction of soil microbiota can strongly influence plant successional trajectory, though their significance in community assembly has been largely overlooked in numerous studies (van der Putten *et al.*, 1993; Kardol *et al.*, 2006; Brooker *et al.*, 2008; Sikes *et al.*, 2012). Microorganisms can either have a positive impact by facilitating nutrients mobilization and uptake, or alter plants performance through competition or pathogenesis (Díaz *et al.*, 1993; Klironomos, 2002; Alberton *et al.*, 2007). The contribution ratio of beneficial versus antagonistic soil organisms remains unclear. However, several studies suggest that it is related to the successional stage of the ecosystem (Janos, 1980; van der Putten *et al.*, 2013), in a way that a positive effect prevails in early successional communities, while later stages are linked to a negative plant-soil feedback (van der Putten *et al.*, 1993; Reynolds *et al.*, 2003).

The present study provides a contribution to the current knowledge about plant-soil feedback in terrestrial ecosystem development by analyzing the growth rate of plant species following mycorrhizal and non-mycorrhizal symbiotic strategies on the northern coast of Funen (Denmark) and on small surrounding sedimentary islands as representative ecosystems of a successional gradient. With this experiment I intend to prove the hypothesis that soil microbiota have a positive impact on plants performance during early succession, while in later stages pathogens have a stronger effect than beneficial microorganisms.

3. AIMS

The aims of the current study are the following:

1. Analyze plant performance in soils of different successional age taken from a coastal ecosystem, in relation to plant symbiotic strategy.
2. Assess if beneficial soil microorganisms have a stronger effect on plant succession than the antagonistic microbiota potentially accumulated over time.
3. Identify which abiotic factors are most related to ecosystem development and community assembly in a sedimentation coast.
4. Contribute to the evidence of the role of soil microorganisms in plant community assembly.

4. ARBUSCULAR MYCORRHIZA AND SOIL-BORNE PATHOGENS

Among all the beneficial soil organisms, arbuscular mycorrhizal fungi deserve special attention, as their association with plant roots, first described at the end of the 19th century, is a very common and widespread type of symbiosis. In fact, more than 80% of terrestrial plant species form arbuscular mycorrhiza (Paszkowski *et al.*, 2002; Smith & Read, 2008; Varma, 2008; Bonfante & Genre, 2010; Moora, 2014; van der Heijden, 2015). Furthermore, the evolution of vascular plants has been marked by arbuscular mycorrhiza. It represents an ancient symbiosis, with records from the early Devonian (Smith & Read, 2008; Varma, 2008). Redecker *et al.* (2000) also reported fossilized fungal hyphae and spores from the Ordovician that could have been a primitive type of this symbiosis.

Arbuscular mycorrhizal fungi are all obligate symbionts belonging to the division of Glomeromycota (Schüßler *et al.*, 2001; Moora, 2014). Based on environmental ribosomal DNA sequences, there could be up to 1600 arbuscular mycorrhiza fungal species. But the number of plants species forming this symbiosis - with facultative and obligate mycorrhizal representatives - reaches to 200000, which means there is a very low host specificity (Brundrett, 2009; Moora, 2014; van der Heijden *et al.*, 2015).

This symbiosis is defined as an endophytic type of mycorrhiza, characterized by having internal organs. Besides the root and the extraradical mycelium, arbuscular mycorrhizae are composed by fungal structures (Figure 1), which are fundamentally vesicles, coils and hyphae, that invaginate on the host cell developing highly branched structures called arbuscules, increasing this way its surface (Smith & Read, 2008; Varma, 2008).

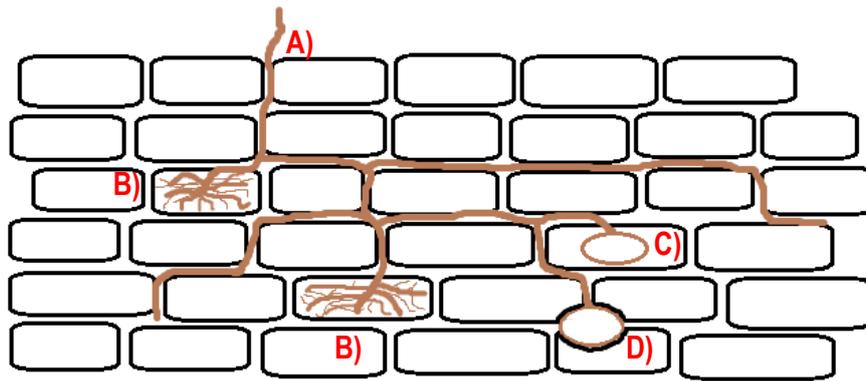


Figure 1. Schematic picture representing mycorrhizal colonization in a root. Plant cells are represented in black. Fungal structures are represented in brown. **A)** Hyphae. **B)** Arbuscules. **C)** Intracellular vesicle. **D)** Intercellular vesicle.

The fungi, which are the heterotrophic component of this symbiosis, provide principally phosphorus acquired by the extraradical fungal hyphae to the plant, and get back the carbohydrates produced by photosynthesis (Varma, 2008). Besides this nutrients exchange, it is known that arbuscular mycorrhizal fungi can strongly influence plant performance in many other different ways. They can increase plant growth and reproductive capacity, as well as their resistance to abiotic stresses (Lu & Koide, 1994; Newsham *et al.*, 1994; Subramanian *et al.*, 1995; Gupta & Kumar, 2000; Smith & Read, 2008). These fungi also protect roots from soil pathogens through competitive and antagonistic interactions (Perrin, 1990; van der Putten *et al.*, 1993; Gange & West, 1994; Azcón-Aguilar & Barea, 1996). However, their effect differs strongly with plant individuals and they can even act as pathogens by generating negative feedbacks on plant growth (Johnson, Graham & Smith, 1997; Kiers *et al.*, 2001; Bever, 2002; Castelli & Casper, 2003; van der Putten *et al.*, 2013).

Soil-borne pathogens belong to three groups: bacteria, nematodes and fungi. They grow and penetrate the roots once they get in contact with root exudates, sometimes after remaining in the soil for a long period. Some of the symptoms that plants express are root rot or blackening, wilt, stunting and even host death. Afterwards pathogens can move to another root and infect it. Their effect can be devastating on crops (Newhook & Podger, 1972; Matson & Warning, 1984; Carlsson *et al.*, 1990; Haas & Défago, 2005). Nevertheless, they also contribute to the succession of plant species, as they seem to affect particularly some phases of the population development (van der Putten *et al.*, 1993; Carlsson *et al.*, 1990). They are plant-specific and do not affect the growth of all species in the same way. This different sensitiveness of plants is thought to be an evolutionary response to selection pressures of the succession stage, where also interspecific competition may be enforced (van der Putten *et al.*, 1993; Klironomos, 2003; van der Putten, 2003; van der Putten *et al.*, 2013). It seems that soil-borne pathogens have also a considerable effect on the performance

of above-ground herbivorous insects and natural enemies, as well as on plant community diversity and productivity (Bardgett & Wardle, 2010; de Kroon *et al.*, 2012).

5. MATERIAL AND METHODS

5.1 STUDY AREA

The study was carried out in the northern coast of Funen, the third largest island of Denmark. I examined three zones: two in Bogense and one in Odense. On each site I studied two islands and one point of the mainland (Appendix 1). One of the locations in Bogense presented a marshy soil composed of silt and clay, while the other two zones were sandy, with gravel and coarser sediments. By using aerial photographs of the Danmarks Miljøportal database recorded during last century, the age of each island, which ranges from 19 to 54 years, was determined. I assigned the age of 100 years old to the mainland points, to standardize that it is an older system than the islands. Appendix 2 shows the abiotic data of each examined point, provided by Jespersen *et al.* 2018. The vegetation varies along the successional gradient, with *Salicornia* sp. and *Plantago maritima* being characteristic of young islands, while older ecosystems are dominated by *Artemisia alba*, *Festuca rubra*, *Tripolium pannonicum* and *Elytrigia repens*. Also some species are more representative of sandy areas, such as *Leymus arenarius*, *Tripleurospermum maritimum* and *Atriplex littoralis*. In these ecosystems the vegetation has a clumped distribution, whereas marshy zones have in general a complete vegetation cover with characteristic species like *Puccinellia maritima*.

5.2 SAMPLING DESIGN

The sampling was conducted in early May 2018. In the islands, I took soil from a spot with vegetation in the center, as it represents the age of the island, while the tidal zone is younger. In each sampling site, soil was taken from 8 points two meters apart from each other. After removing stones, shells and roots from the samples, they were stored at 4 °C until the experiment was set up.

Ramets of two local non-mycorrhizal species (*Honckenya peploides* and *Sedum acre*) were also collected from two islands (Bogense 3 and Bogense 7). These were washed and kept at 15 °C with dampened paper until the experiment set-up.

5.3 EXPERIMENTAL SET UP

I established 12 treatments (9 sites plus 3 controls). Each control was a mixed of the soil of the three examined points in a location. They were sterilized by heating them at 69 °C for 12 hours three times.

Commercial relatives of the local species were sown (see Appendix 3). As I was interested in the species response according to their symbiotic strategy, I chose two grasses (mycorrhizal species), two legumes (mycorrhizal species with *Rhizobium* symbiosis for nitrogen fixation) and two representative species of a non-mycorrhizal strategy. The following species were used:

- Grasses (Poaceae): *Lolium perenne* and *Sorghum vulgare*
- Legumes (Fabaceae): *Trifolium alexandrinum* and *Lotus corniculatus*
- Non-mycorrhizal species: *Sinapis alba* (Brassicaceae) and *Spinacia oleracea* (Amaranthaceae)

I had two replicates of each species per treatment, and I sowed three seeds per pot. Since not all of them survived, after two weeks only one individual was kept per pot to standardize the competition level for all the plants. I also removed plants that were spontaneously growing due to spores or propagules that remained in the soil samples.

The collected ramets were also planted following the same set-up.

5.4 PLANT GROWTH RATE AND MYCORRHIZAL COLONIZATION MEASUREMENTS

After six weeks growing in the greenhouse, the plants were washed and weighed. The above- and below-ground weight was separately measured. Then they were dried at 70 °C for 48 hours and weighed again to calculate the water content. Half of the roots were kept in 50% ethanol and stained following the ink-vinegar protocol (Vierheilig *et al.*, 1998) to visualize the mycorrhizal structures. The percentage of hyphal, arbuscular and vesicular colonization was quantified under a compound light microscope at 10-40x following the intersection method from McGonigle *et al.* (1990). All the calculations were performed with Microsoft Excel 2013.

5.5 STATISTICS

For the statistical analysis I employed the R statistics version 3.4.4. I conducted an ANOVA and Bonferroni post-hoc analyses with the age of the ecosystems and the final weight of the plants (above-, below-ground and total weight). To do that I plotted the age of each site in three categories: new (1), medium age (2) and old systems (3). I performed this analysis for all plant species together, as well as for each of the six commercial species separately. The data of each of the three locations

were also separately evaluated. The percentage of mycorrhizal colonization and the abiotic factors were examined with the same procedure. I used boxplots to represent the results.

Also a linear mixed model with the final weight as the response and the age as the effect variable was conducted, where the zone, the symbiotic strategy and the species were used as random factors.

6. RESULTS

Appendix 3 shows the survival rate and percentage of water content of the studied species.

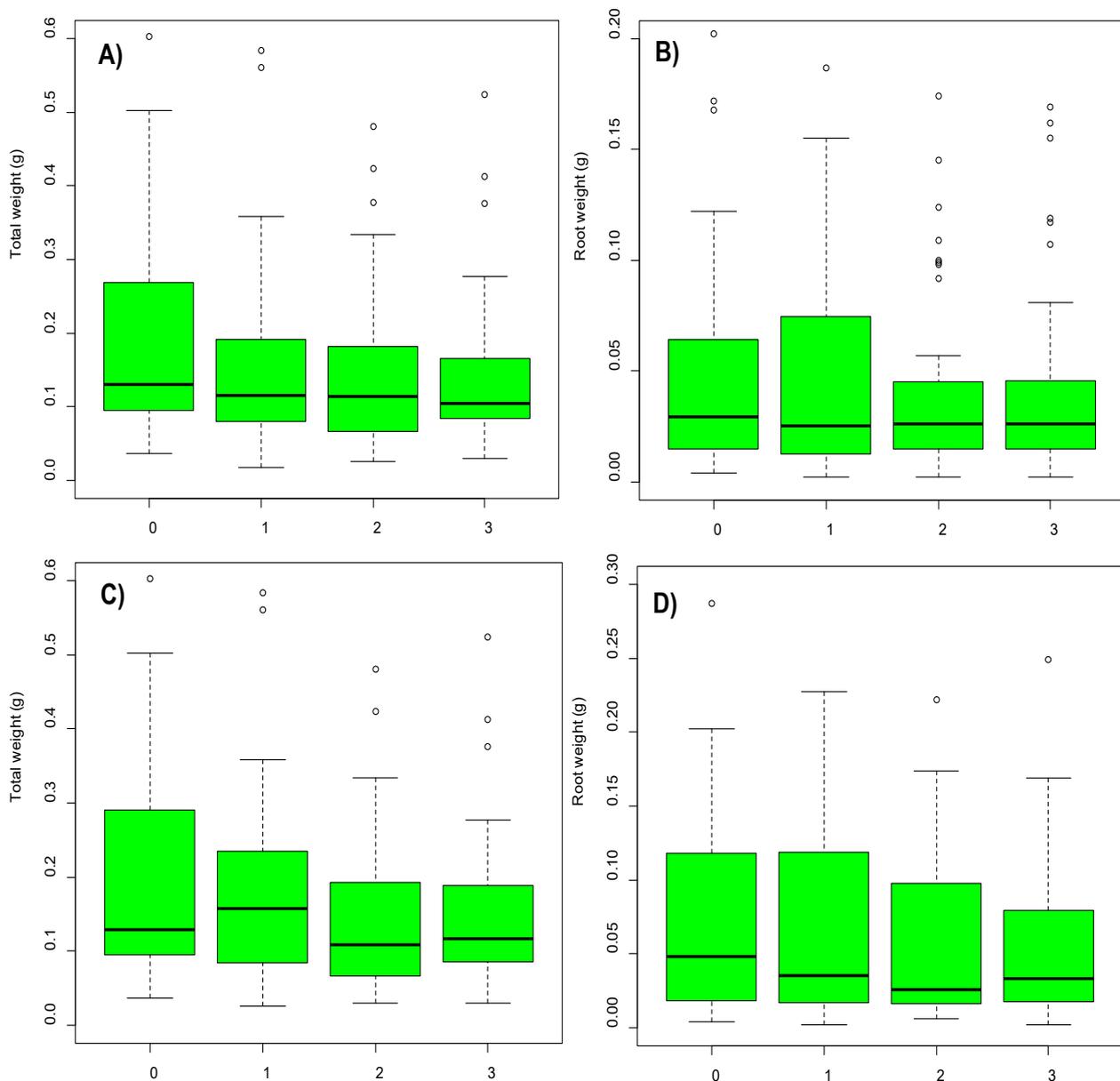


Figure 2. Boxplots representing total weight (A and C) and below-ground weight (B and D) in the Y axis, and age factors as the independent variable. **A)** and **B)** represent the data of seeds and ramets. **C)** and **D)** represent the data of the seeds only.

When conducting an ANOVA and post-hoc analysis with the age factor and the total weight, there is only significant difference between the control (age 0) and the other age groups. However, the boxplots show a tendency of plant species to have a higher growth rate in recently settled ecosystems than in older ones (Figure 2).

If I perform separate models for each of the three locations, the results obtained with the data from the marshy soils in Bogense seem to follow this trend more distinctly than the other two zones (Figure 3), but still with no significant difference between the three age groups.

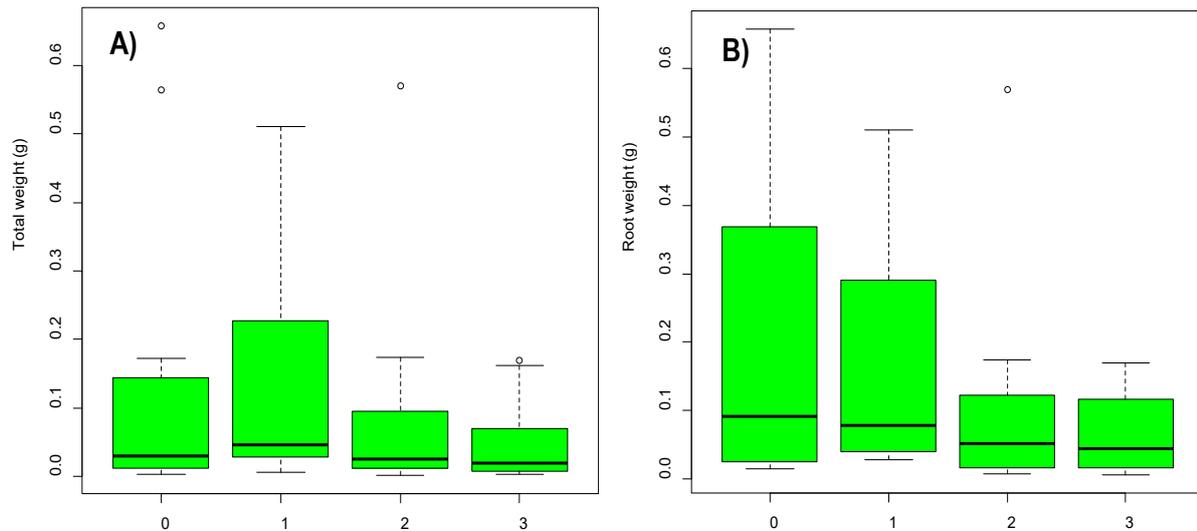


Figure 3. Boxplots representing the data of marshy soils. The graphics depict final plant weight in the Y axis, and age factors as the independent variable. **A)** represents total weight data from seeds and ramets. **B)** represents below-ground weight data from seeds only.

I plotted the age of each study site as a continuous variable with the six commercial species separately, and I found there is a big difference in their performance. As we can see in Figure 4, the two grasses *Lolium perenne* and *Sorghum vulgare* show a decrease in growth rate when the age of the ecosystem is higher, which is also noticeable for the two non-mycorrhizal forbs *Sinapis alba* and *Spinacia oleracea*. By contrast, the two legumes *Trifolium alexandrinum* and *Lotus corniculatus* have no clear pattern. This is appreciable for the data of the three locations. Nevertheless, the mixed model only showed a significant effect of this linear relationship when the age was used as a three-levels factor.

About the mycorrhizal colonization (Figure 5), it was found, as expected, that the old ecosystems presented a higher percentage, followed by the sites with medium age soil (Figure 6a). What is also visible from this data is that marshy soils were more colonized than sandy ones (Figure 6b).

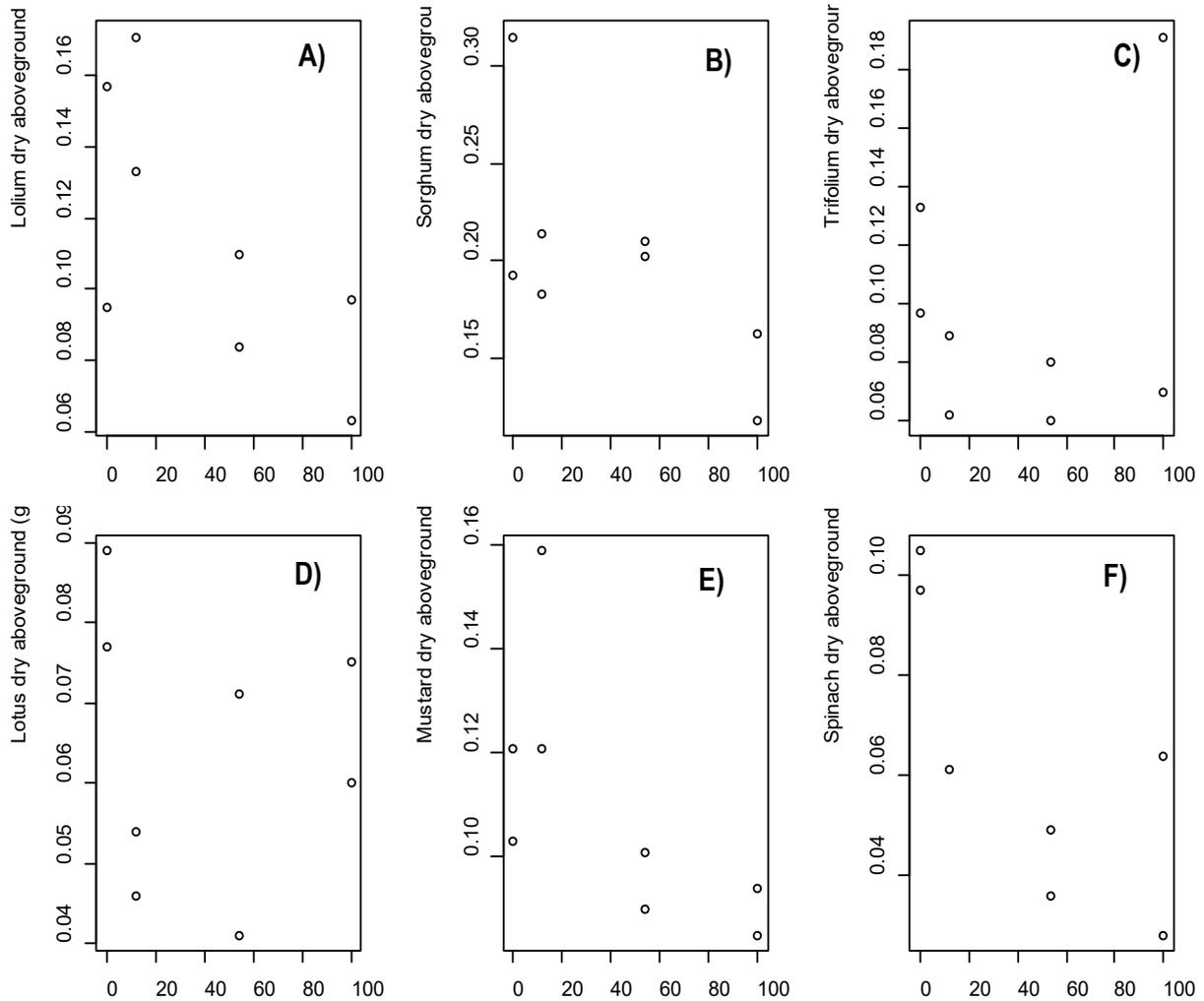


Figure 4. Graphics representing the above-ground weight data of sandy soils in Bogense for each seeds species separately. The age of each sample site is represented in the X axis as a continuous variable. **A)** *Lolium perenne*. **B)** *Sorghum vulgare*. **C)** *Trifolium alexandrium*. **D)** *Lotus corniculatus* **E)** *Sinapis alba* **F)** *Spinacia oleracea*.

Finally, two of the abiotic variables were found to be positively related to the total weight of the plants: percentage of organic material and water content (Figure 7a). These two factors were significantly higher in marshy soils (Figure 7b).

7. DISCUSSION

The results obtained in this experiment show tendencies of plant-soil feedbacks change during the course of primary succession. These results may contribute to a deeper understanding of how biotrophic fungi interact with plants during community assembly.

It has generally been found that, in young ecosystems – such as newly established islands – the pressure of soil-borne pathogens is lower than in older ecosystems, where their abundance has accumulated over time (Carlsson & Elmqvist, 1992; Carlsson-Granér, 1997). My results are

aligned with these findings (Figure 2), although observed indirectly through performance of plants with little or no dependence on mycorrhiza and therefore a more direct response to soil-borne diseases. Arbuscular mycorrhizal fungi have similarly been found to accumulate with ecosystem age (Johnson *et al.*, 1991; Hart *et al.*, 2001), which is to be expected from obligate biotrophic fungi, which must depend on colonizing plants. I observed a similar increase in arbuscular mycorrhiza colonization rate for all study sites (Figure 6a). Therefore, I suggest that, overall, during early successional stages mutualist microorganisms may have a stronger effect on plants performance than pathogens, resulting in a positive plant-soil feedback, opposite to what occurs in later stages.

It has been postulated that the intensity of pathogen pressure varies through time (Smith *et al.*, 2011, Ericson *et al.*, 2016), and that, together with mycorrhizal fungi, pathogens are important drivers of succession (Janos, 1980; Read, 1991; van der Putten *et al.*, 1993; Reynolds *et al.*, 2003). However, there is controversy about in which direction. While part of the scientific community suggests that the positive effect of mycorrhizal fungi is stronger than the negative feedback generated by pathogens (Kardol *et al.*, 2006; Sikes *et al.*, 2012; Uibopuu *et al.*, 2012), other researchers propose the opposite hypothesis, with a negative plant-soil feedback prevailing in later successional stages, coinciding with the tendency observed in this experiment: Van der Putten *et al.* (1993) reported that plant individuals in a foredune community showed a reduced biomass when planted in the soil of their successors, but not in the soil of their predecessors. Reynolds *et al.* (2003) also suggested that positive plant-soil feedback is more important during early successional communities, contrary to later stages, where negative feedback is more significant. However, there is a wide variety of factors shaping this tendency, some of which I describe here.

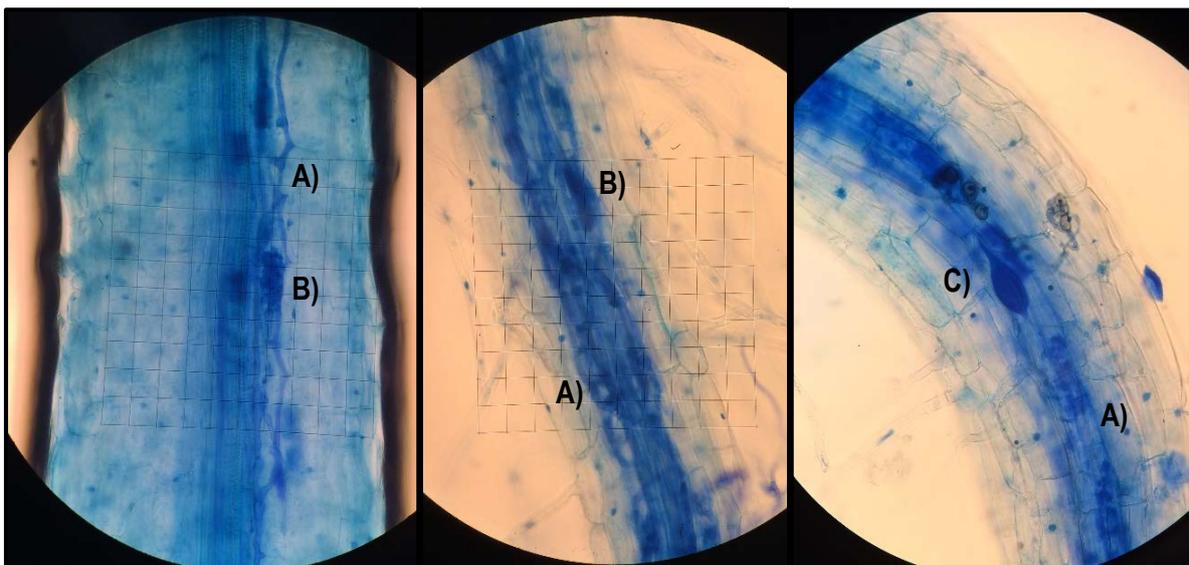


Figure 5. Microscopy pictures of colonized roots where fungal structures are visible. **A)** Hyphae. **B)** Arbuscules. **C)** Vesicle.

In the current study, the pattern of soil feedback was more clearly followed in marshy soils (Figure 3). Taking into account that the percentage of soil organic material and soil water content of these sites were higher (Figures 7c and 7d), and that these factors were the most correlated abiotic factors with plant growth (Figure 7a and 7b), I propose that ecosystems where the amount of organic material and/or water accumulation capacity is high may have higher rates of beneficial soil microorganisms. This hypothesis is also supported by the outcome of mycorrhizal colonization percentage, which was higher in marshy soils compared to sandy areas (Figure 6b). Hudson (1994)

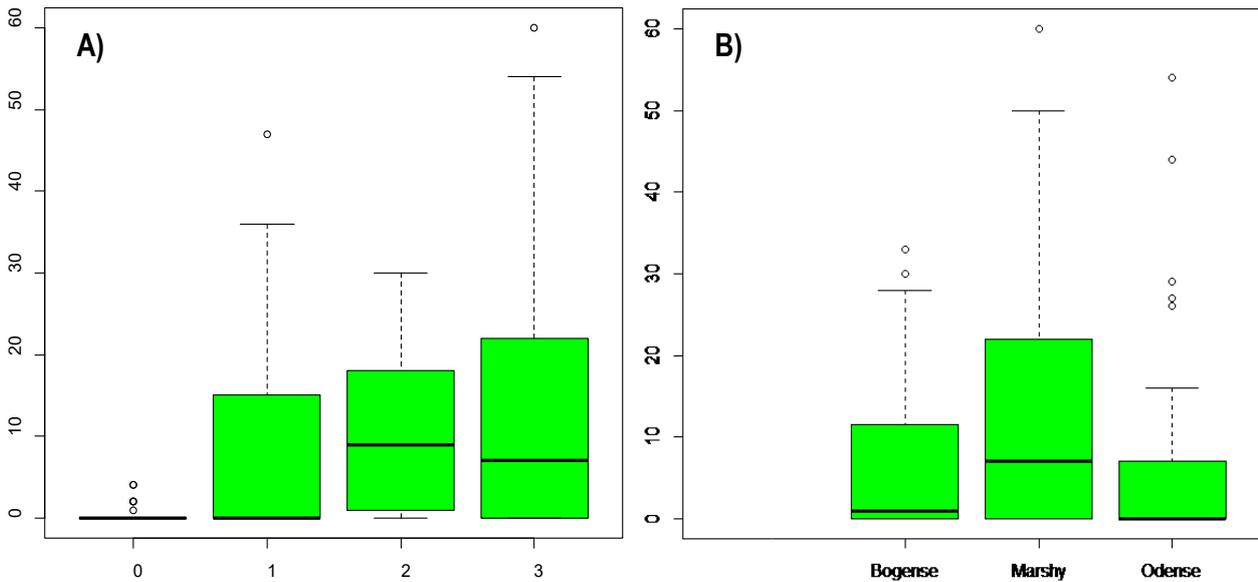


Figure 6. Boxplots representing the percentage of mycorrhizal colonization in the Y axis, and the age factors (A) and the zone (B) as the independent variable. *Bogense* represents the sandy areas of this location.

found that organic matter content and water capacity of the soil are two environmental variables positively correlated. Regarding all this, the fact that during primary succession the water availability is higher than in later stages is related to a reduced plant susceptibility to fungal pathogens (Gates *et al.*, 1986; Paul & Ayres, 1986; Carlsson *et al.*, 1990). It has also been stated that the variation of precipitation, among other environmental factors, affect epidemic development so that drier locations present a higher disease rate (Newhook & Podger, 1972; Palti, 1981; Ingvarsson & Ericson, 2000; Thrall *et al.*, 2001; Laine & Hanski, 2006; Alexander *et al.*, 2007; Ericson *et al.*, 2016). It seems then that soil water content, and thus percentage of organic matter, are two crucial abiotic factors in plant-soil feedbacks.

When conducting separate analyses for each experimental plant species, the observed tendency is appreciable especially for grasses (Figures 4a and 4b) and also for non-mycorrhizal species (Figures 4e and 4f). In contrast, legumes do not show a clear pattern (Figures 4c and 4d).

These variations among species with different symbiotic strategies could mean that the sensitiveness of arbuscular mycorrhizal plants (excluding legumes) to the presence of pathogens is higher, as it cannot be compensated even when the abundance of their mutualistic symbionts is high, resulting in a decline of their growth rate in old ecosystems. In relation to this, Bennet *et al.* (2017) found that plants with arbuscular mycorrhizal association experience negative soil feedbacks compared to other types of symbiosis. Even though arbuscular mycorrhiza formation could be effective against pathogens, this does not happen under all circumstances (Azcón-Aguilar & Barea, 1996). Non-mycorrhizal plants showed also some tendency, but they are independent of

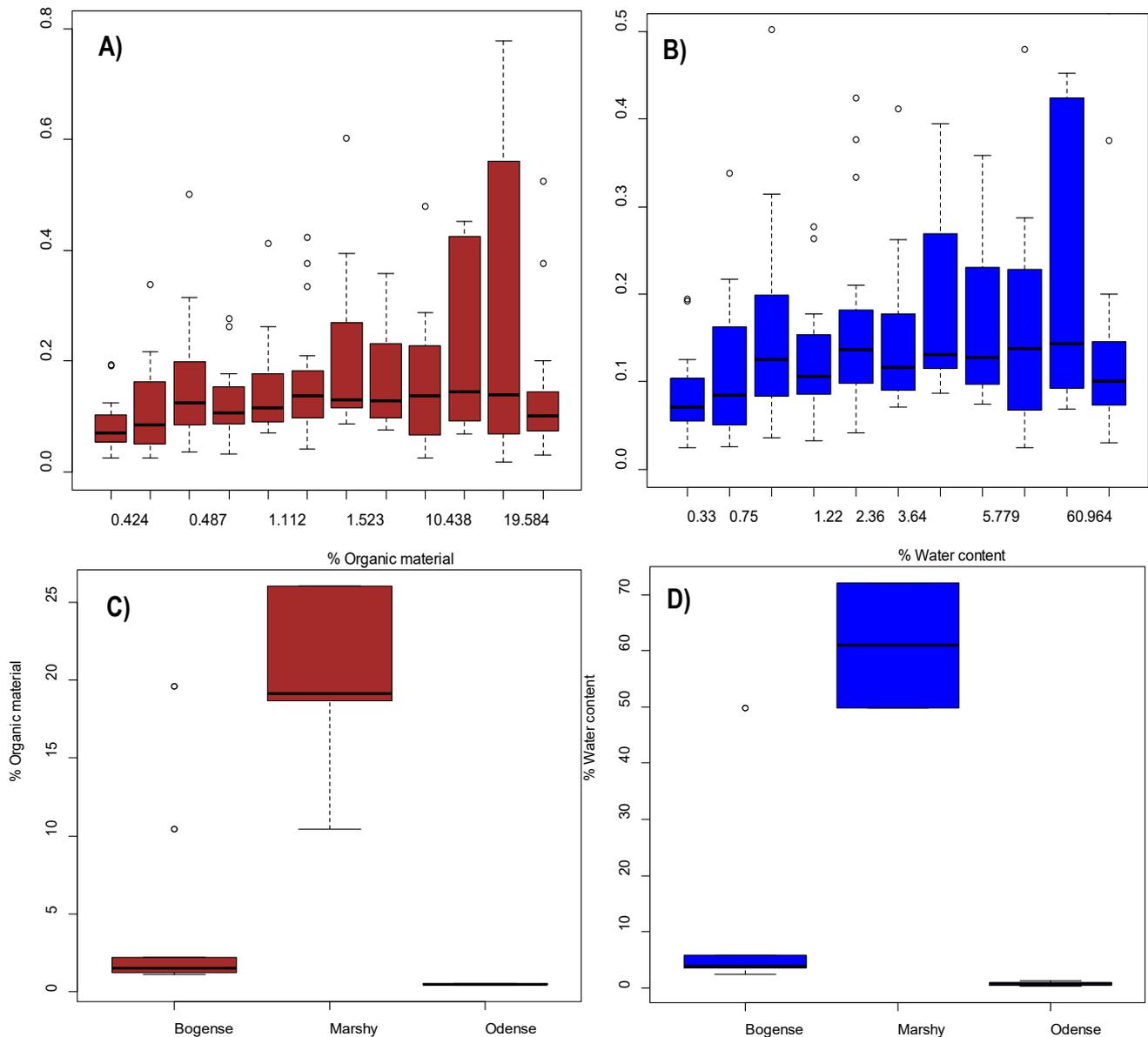


Figure 7. Boxplots representing the abiotic factors. **A)** and **B)** depict the percentage of organic material and water content, respectively, in relation to the total weight (g) of plants. **C)** and **D)** represent these variables in each zone separately. *Bogense* refers to the sandy areas of this location.

arbuscular mycorrhizal fungi, so they are clearly disadvantaged in old ecosystems, as the accumulation of beneficial microorganisms does not affect them. About legumes, they seem to be less sensitive to antagonistic soil microorganisms, since their growth rate does not decrease in old ecosystems despite the elevated proportion of soil-borne pathogens. This could be due to their association with *Rhizobium* for nitrogen fixation, which definitely favors their performance. All these differences in the performance of the studied plant species could help to understand the development of the plant community over successional time. It could be expected that non-mycorrhizal species dominate early successional stages, as they are not adversely affected by the low abundance of mycorrhizal fungi, in contrast to mycorrhizal species. Janos *et al.* (1980) already postulated a shift from non-mycorrhizal to obligated mycorrhizal species along the successional gradient. In this study, however, mycorrhizal species showed a different behavior in late succession: legumes succeeded, but the growth rate of grasses declined. This pattern is contradictory to the idea that legumes, as ecosystem engineers, should be especially important during early colonization, since they are essential constituents of the soil building process through nitrogen fixation (Chapin *et al.*, 1994; Larson & Siemann, 1998; Reynolds *et al.*, 2003). In relation to this, in my greenhouse experiment I could not take into account several important aspects that should be considered in the study of plant community assembly, and that deserve to be mentioned. First of all, there is a variety of other plant features related to the development of the community over time: the quality and quantity of anti-herbivore defense, for example, differs in early and late successional species, with a higher specialization over time (Reynolds *et al.*, 2003); shorter-lived plants experience higher disease transmission rates than longer-lived host species (Carlsson-Granér & Thrall, 2006); also herbaceous plants seem to dominate early stages in sand dunes, whereas woody plants are more representative of later stages (Reynolds *et al.*, 2003). In addition, the size of the community plays a key role in its development over time, since inter- and intraspecific competition strongly affects the performance of plant individuals. In relation to soil-borne pathogens, host population size influences community density of antagonistic microorganisms (Newhook & Podger, 1972; Antonovics & Levin, 1980; Carlsson *et al.*, 1990). There is even a minimum threshold of host population size below which the presence of pathogens is highly unlikely (Ericson *et al.*, 2016). On the other hand, it is known that during the phase of early colonization there is a low genetic heterogeneity within the community (Nei *et al.*, 1975) that results in an increased susceptibility to pathogen infections (Dinoor & Eshed, 1984; Carlsson *et al.*, 1990).

Together with time, space is one of the most important variables during ecological and evolutionary interactions between pathogens and their hosts (Frank, 1991; Thrall & Burdon, 1999; Brooks *et al.*, 2008; Carlsson-Granér *et al.*, 2014; Ericson *et al.*, 2016). Previous studies considered

the spatial structure, and it was stated that small isolated populations are less likely to have specialist pathogens (because of limited dispersal), making their ecological and evolutionary trajectory different from communities that are more connected (Carlsson *et al.*, 1990; Hanski & Ovaskainen, 2000; Alexander *et al.*, 2007; Carlsson-Granér *et al.*, 2014). The islands examined in this study are a representation of small isolated ecosystems (especially the sandy locations, since marshy islands are closer to each other), where the pathogen dispersal is slow, resulting in a low disease rate (Burdon *et al.*, 1989; Thrall & Antonovics, 1995; Carlsson-Granér & Thrall, 2002; Carlsson-Granér *et al.*, 2014). In fact, when I performed the quantification of mycorrhizal colonization, in general I did not find signs of pathogenesis in the roots.

As described in this study, there are multiple factors affecting the ecological development of the study system. Nevertheless, the occurrence of soil-borne diseases may be of great importance in the natural succession of ecosystems with similar characteristics (sedimentary islands). Plant species to be replaced in later successional stages may be disfavored by pathogens, whereas succeeding species must be tolerant, and thus have a competitive advantage, as described by the inhibition model of Connell & Slatyer (1977), where early colonists would be replaced by other species only after they are damaged or killed (for instance, by pathogenesis), but not because of competitive interactions for resources between plant species directly. Ecological and evolutionary processes shaping species distributions have become a very important topic and recently gained more interest of the scientific community due to its relevance in current and concerning issues such as climate change and the biodiversity crisis.

8. CONCLUSIONS

1. I studied a sedimentation coast in Funen (Denmark) and small arisen islands surrounding it. My main interest was to contribute to the understanding of the role of beneficial and detrimental soil microorganisms in primary succession. I carried out the experiment in spring 2018.
2. A higher plant growth rate was observed in recently arisen islands (where the soil microbial community was still young and small) compared to older ecosystems (with an assumed higher colonization of soil microorganisms). This observed pattern differed when looking at species separately.
3. Within mycorrhizal plant species, grasses showed a higher sensitiveness to the effect of the age of the system and a lower dependence to the presence of arbuscular mycorrhizal fungi than legumes.

4. Non-mycorrhizal plant species also seem to be sensitive to the effect of antagonistic microorganisms accumulated in the soil over time.
5. Legumes had no pattern, probably because the accumulation of bacterial symbionts over time can compensate the detrimental effect of pathogen accumulation.
6. Some abiotic factors appear to be more important than others influencing the growth rate pattern of plants. The percentage of organic matter and the percentage of water content of the soil seem to be more closely related to plant growth. The values of these two factors were significantly higher in marshy soils than in sandy soils. Marshy systems also presented a higher percentage of mycorrhizal colonization.
7. I found that the symbiotic strategy of plant species is related to their establishment in a natural ecosystem, and it is an important factor shaping the community system along the successional gradient.
8. Soil pathogens and mycorrhizal fungi play a fundamental role in primary succession and should be taken into account in future investigations of plant community assembly.

9. FUTURE RESEARCH PERSPECTIVES

- ✓ It is necessary to carry out annual experiments in the same study system to evaluate the potential effect of environmental variables differing over the years.
- ✓ It is convenient to sample a higher number of islands and mainland points to have more representative and significant outcomes.
- ✓ It would be recommendable to study more native species of the study area to have a closer idea about their performance.
- ✓ It would be interesting to develop a field experiment where the competitive interaction of species and other community aspects can be considered.

10. REFERENCES

- Alberton, O., Kuyper, T. W. & Gorissen, A. (2007). Competition for nitrogen between *Pinus sylvestris* and ectomycorrhizal fungi generates potential for negative feedback under elevated CO₂. *Plant Soil* 296: 159-172.
- Alexander, H. M., Price, S., Houser, R., Finch, D. & Tourtellot, M. (2007). Is there reduction in disease and pre-dispersal seed predation at the border of host plant's range? Field and herbarium studies of *Carex blanda*. *Journal of Ecology* 95: 446-457.

- Antonovics, J. & Levin, D. A. (1980). The ecological and genetic consequences of density-dependent regulation in plants. *Annual Review of Ecology and Systematics* 11: 411-452.
- Azcón-Aguilar, C. & Barea, J. N. (1996). Arbuscular mycorrhizas and biological control of soil-borne plant pathogens – an overview of the mechanisms involved. *Mycorrhiza* 6: 457-464.
- Bardgett, R. D. & Wardle, D. A. (2010). *Aboveground-Belowground Linkages: Biotic Interactions, Ecosystem Processes and Global Change*. Oxford University Press, New York. 301 pp.
- Bennet, J. A., Maherali, H., Reinhart, K. O., Leckberg, Y., Hart, M. M. & Klironomos, J. (2017). Plant-soil feedbacks and mycorrhizal type influence temperate forest population dynamics. *Science* 355: 181-184.
- Bever, J. D. (2002). Negative feedback within a mutualism: host-specific growth of mycorrhizal fungi reduces plant benefit. *Proc. R. Soc. Lond. B* 269: 2595-2601.
- Bonfante, P. & Genre, A. (2010). Mechanisms underlying beneficial plant-fungus interactions in mycorrhizal symbiosis. *Nature Communications* 1:48.
- Brooker, R. W., Maestre, F. T., Callaway, R. M., Lortie, C. L., Cavieres, L. A., Kunstler, G., Liancourt, P., Tielbörger, K., Travis, J. M. J., Anthelme, F., Armas, C., Coll, L., Corcket, E., Delzon, S., Forey, E., Kikvidze, Z., Olofsson, J., Pugnaire, F., Quiroz, C. L., Saccone, P., Schiffers, K., Seifan, M., Touzard, B. & Michalet, R. (2008). Facilitation in plant communities: the past, the present, and the future. *Journal of Ecology* 96: 18-34.
- Brooks, C. P., Antonovics, J. & Keitt, T. H. (2008). Spatial and Temporal Heterogeneity Explain Disease Dynamics in a Spatially Explicit Network Model. *The American Naturalist* 172(2): 149-152.
- Brundett, M. C. (2009). Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants resolving conflicting information and developing reliable means of diagnosis. *Plant Soil* 320:37-77.
- Burdon, J. J., Jarosz, A. M & Kirby, G. C. (1989). Pattern and patchiness in plant-pathogen interaction: causes and consequences. *Annual Review of Ecology and Systematics* 20: 119-136.
- Carlsson-Granér, U. (1997). Anther-smut disease in *Silene dioica*: variation in susceptibility among genotypes and populations and patterns of disease within populations. *Evolution* 51: 1416-1426.
- Carlsson, U. & Elmqvist, T. (1992). Epidemiology of anther-smut disease (*Microbotryum violaceum*) and numeric regulation of populations of *Silene dioica*. *Oecologia* 90: 509-517.

- Carlsson-Granér, U. & Thrall, P. H. (2002). The spatial distribution of plant populations, disease dynamics and evolution of resistance. *Oikos* 97: 97-110.
- Carlsson-Granér, U. & Thrall, P. H. (2006). The impact of host longevity on disease transmission: host-pathogen dynamics and the evolution of resistance. *Evolutionary Ecology Research* 8: 1-17.
- Carlsson, U., Elmqvist, T. Wennström, A. & Ericson, L. (1990). Infection by pathogens and population age of host plants. *Journal of Ecology* 78: 1094-1105.
- Carlsson-Granér, U., Giles, B. E. & Thrall, P. H. (2014). Patterns of disease and host resistance in spatially structured systems. *European Journal of Plant Pathology* 138: 499-511.
- Castelli, J. P. & Casper, B. B. (2003). Intraspecific AM fungal variation contributes to plant-fungal feedback in a serpentine grassland. *Ecology* 84(2): 323-336.
- Chapin, F. S., Walker, L. R., Fastie, C. L. & Sherdman, L. C. (1994). Mechanisms of primary succession following deglaciation at Glacier Bay, Alaska. *Ecological Monographs* 64(2): 149-175.
- Connell, J. H. & Slatyer, R. O. (1977). Mechanisms of succession in natural communities and their role in community stability and organization. *The American Naturalist* 111(982): 1119-1144.
- De Kroon, H., Hendriks, M., van Ruijven, J., Ravenek, J., Padilla, F. M., Jongejans, E., Visser, E. W. & Mommer, L. (2012). Root responses to nutrients and soil biota: drivers of species coexistence and ecosystem productivity. *Journal of Ecology* 100: 6-5.
- Díaz, S., Grime, J. P., Harris, J. & McPherson, E. (1993). Evidence of a feedback mechanism limiting plant response to elevated carbon dioxide. *Nature* 364: 616-617.
- Dinoor, A. & Eshed, N. (1984). The role and importance of pathogens in natural plant communities. *Annual Review of Phytopathology* 22: 443-466.
- Ericson, L., Müller, W. J. & Burdon, J. J. (2016). 28 year temporal sequence of epidemic in a natural rust – host plant metapopulation. *Journal of Ecology* 105(3): 701-713.
- Frank, S. A. (1991). Ecological and genetic models of host-pathogen coevolution. *Heredity* 67: 73-83.
- Gange, A. C. & West, H. M. (1994). Interactions between arbuscular mycorrhizal fungi and foliar-feeding insects in *Plantago lanceolata* L. *New Phytologist* 128: 79-87.
- Gates, D. J., Westcott, M., Burdon, J. J. & Alexander, H. M. (1986). Competition and stability in plant mixtures in the presence of disease. *Oecologia* 68: 559-566.

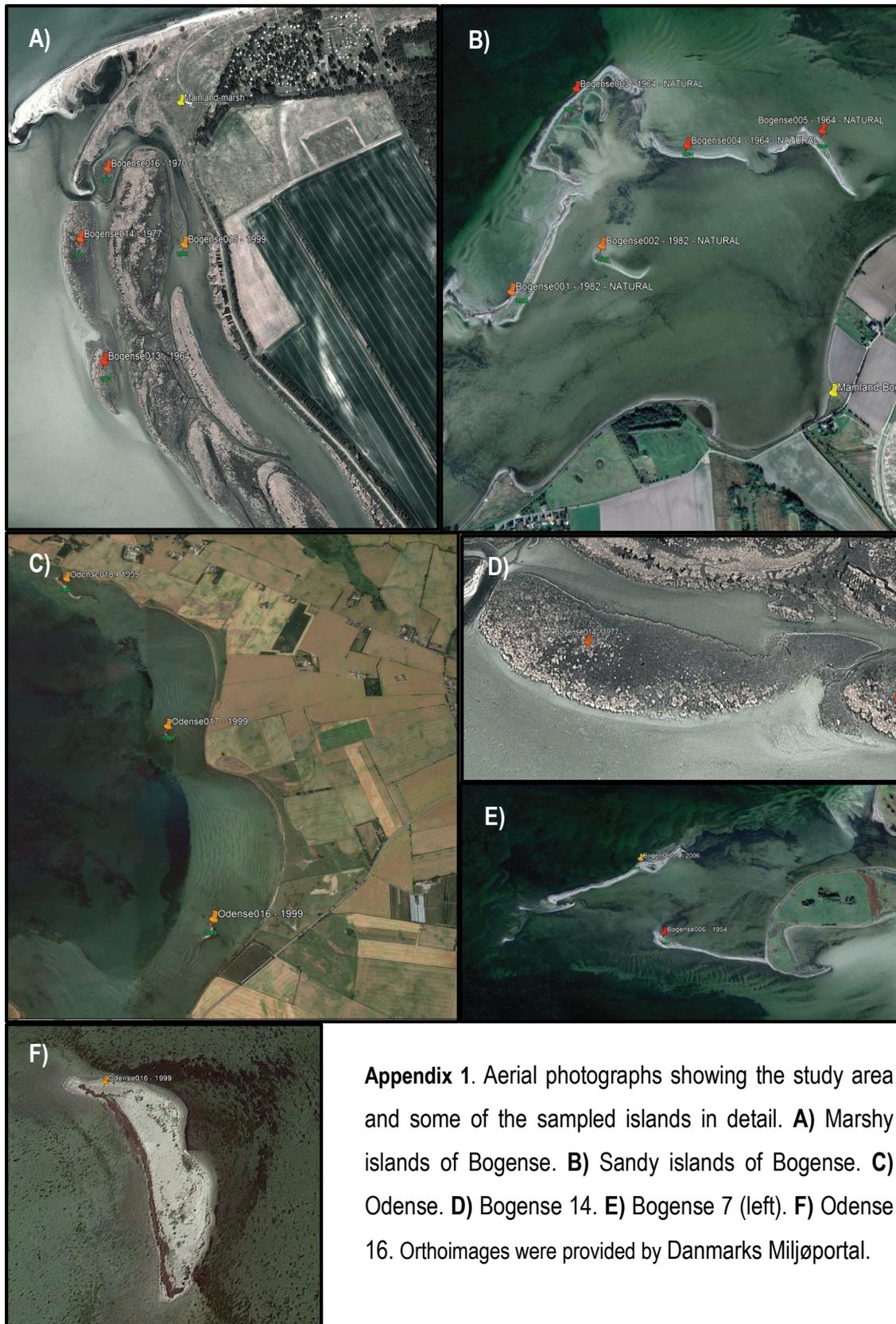
- Gupta, R. K. & Kumar, P. (2000). Mycorrhizal plants in response to adverse environmental conditions. In: *Mycorrhizal Biology*. Plenum Publisher, India, 67-84 pp.
- Haas, D. & Défago, G. (2005). Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nature Review Microbiology*, 3:307-319.
- Hanski, I. & Ovaskainen, O. (2000). The metapopulation capacity of a fragmented landscape. *Nature* 404: 755-758.
- Hart, M. M., Reader, R. J. & Klironomos, J. N. (2001). Life-history strategies of arbuscular mycorrhizal fungi in relation to their successional dynamics. *Mycologia* 93(6): 1186-1194.
- Hudson, B. D. (1994). Soil organic matter and available water capacity. *Journal of Soil and Water Conservation* 49(2): 189-194.
- Ingvarsson, P. K. & Ericson, L. (2000). Exploitative competition between two seed parasites on the common sedge, *Carex nigra*. *Oikos* 91: 362-370.
- Janos, D. P. (1980). Mycorrhizae Influence Tropical Succession. *Biotropica* 12: 56-64.
- Jespersen, J. R. P. (2018). Succession of arbuscular mycorrhiza in a Danish coastal island system. *University of Copenhagen. Master Thesis*.
- Jing, J., Bezemer, T. J. & van der Putten, W. H. (2015). Complementarity and selection effects in early and mid-successional plant communities are differentially affected by plant-soil feedback. *Journal of Ecology* 103: 641-647.
- Johnson, N. C., Zak, D. R., Tilman, D. & Pfleger, F. L. (1991). Dynamics of vesicular-arbuscular mycorrhizae during old field succession. *Oecologia* 86(3): 349-358.
- Johnson, N. C., Graham, J. H. & Smith, F. A. (1997). Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytologist* 135: 575-585.
- Kardol, P., Bezemer, T. M. & van der Putten, W. H. (2006). Temporal variation in plant-soil feedback controls succession. *Ecology Letters* 9: 1080-1088.
- Kiers, E. T., Duhamel, M., Beesetty, Y., Mensah, J. A., Franken, O., Verbruggen, E., Fellbaum, C. R., Kowalchuk, G. A., Hart, M. M., Bago, A., Palmer, T. M., West, S. A., Vandenkoornhuyse, P., Jansa, J. & Bücking, H. (2001). Reciprocal Rewards Stabilize Cooperation in the Mycorrhizal Symbiosis. *Science* 333(6044): 880-882.
- Klironomos, J. N. (2002). Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature* 417:67-70.
- Klironomos, J. N. (2003). Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology* 84(9): 2292-2301.

- Laine, A. & Hanski, I. (2006). Large-scale spatial dynamics of a specialist plant pathogen in a fragmented landscape. *Journal of Ecology* 94: 217-226.
- Larson, J. L. & Siemann, E. (1998). Legumes May Be Symbiont-limited During Old-field Succession. *The American Midland Naturalist* 140(1): 90-95.
- Lu, X. & Koide, R. T. (1994). The effects of mycorrhizal infection on components of plant growth and reproduction. *New Phytologist* 128: 211-218.
- Matson, P. A. & Waring, R. H. (1984). Effects of Nutrient and Light Limitation on Mountain Hemlock Susceptibility to Lamiated Root Rot. *Ecology* 65: 1517-1524.
- McGonigle, T. P., Miller, M. H., Evans, D. G., Fairchild, G. L. & Swan, J. A. (1990). A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist* 115(3): 495-501.
- Moora, M. (2014). Mycorrhizal traits and plant communities: perspectives for integration. *Journal of Vegetation Science* 25: 1126-1132.
- Nei, M., Maruyama, T & Chakraborty, R. (1975). The bottleneck effect and genetic variability in populations. *Evolution* 29: 1-10.
- Newhook, F. J. & Podger, F. D. (1972). The role of *Phytophthora cinnamomi* in Australian and New Zealand forests. *Annual Review of Phytopathology* 10: 299-326.
- Newsham, K. K., Fitter, A. H. & Watkinson, A. R. (1994). Root Pathogenic and Arbuscular Mycorrhizal Fungi Determine Fecundity of Asymptomatic Plants in the Field. *British Ecological Society* 82(4): 805-814.
- Palti, J. (1981). *Cultural practices and infectious crop diseases*. Springer, Berlin. 244 pp.
- Paszkowski, U., Kroken, S., Roux, C. & Briggs, S. P. (2002). Rice phosphate transporters include an evolutionarily divergent gene specifically activated in arbuscular mycorrhizal symbiosis. *PNAS* 99(20): 13324-13329.
- Paul, N. D. Ayres, P. G. A. (1986). The impact of a pathogen (*Puccinia Lagenophorae*) on populations of groundsel (*Senecio vulgaris*) overwintering in the field. *Journal of Ecology* 74: 1085-1094.
- Perrin, R. (1990). Interactions between mycorrhizae and diseases caused by soil-borne fungi. *Soil Use and Management* 6(4): 189-194.
- Read, D. J. (1991). Mycorrhizas in ecosystems. *Experientia* 47: 376-391.
- Redecker, D., Kodner, R. & Graham, L. E. (2000). Glomalean Fungi from the Ordovician. *Science* 289: 1920-1922.

- Reynolds, H. L., Packer, A., Bever, J. D. & Clay, K. (2003). Grassroots ecology: plant-microbe-soil interactions as drivers of plant community structure and dynamics. *Ecology* 84(9): 2281-2291.
- Schüßler, A., Schwarzott, D. & Walker, C. (2001). A new fungal phylum, the *Glomeromycota*: phylogeny and evolution. *Mycological Research* 105(12): 1413-1421.
- Sikes, B. A., Maherali, H. & Klironomos, J. N. (2012). Arbuscular mycorrhizal fungal communities change among three stages of primary sand dune succession but do not alter plant growth. *Oikos* 121: 1791-1800.
- Smith, D. L., Ericson, E. & Burdon, J. J. (2011). Co-evolutionary hot and cold spots of selective pressure move in space and time. *Journal of Ecology* 99: 634-641.
- Smith, S. E. & Read, D. R. (2008). *Mycorrhizal Symbiosis*. Elsevier, London. 803 pp.
- Subramanian, K. S., Charest, C., Dwyer, L. M. & Hamilton, R. I. (1995). Arbuscular mycorrhizas and water relations in maize under drought stress at tasseling. *New Phytologist* 129: 643-650.
- Thrall, P. H. & Antonovics, J. (1995). Theoretical and empirical studies of metapopulations- population and genetic dynamics of the *Silene-Ustilago* system. *Canadian Journal of Botany* 73(S1): 1249-1258.
- Thrall, P. H. & Burdon, J. J. (1999). The spatial scale of pathogen dispersal: Consequences for disease dynamic and persistence. *Evolutionary Ecology Research* 1, 681-701.
- Thrall, P. H., Burdon, J. J. & Bock, C. H. (2001). Short-term epidemic dynamics in the *Cakile maritima*-*Alternaria brassicicola* host-pathogen association. *Journal of Ecology* 89: 723-735.
- Uibopuu, A., Moora, M., Öpik, M. & Zobel, M. (2012). Temperate forest understorey species performance is altered by local arbuscular mycorrhizal fungal communities from stands of different successional stages. *Plant Soil* 356: 331-339.
- Van der Heijden, M. G. A., Martin, F. M., Selosse, M. & Sanders, I. R. (2015). Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytologist* 205: 1406-1423.
- Van der Putten, W. H. (2003). Plant defense belowground and spatiotemporal processes in natural vegetation. *Ecology* 84(9): 2269-2280.
- Van der Putten, W. H., van Dijk, C. & Peters, B. A. M. (1993). Plant-specific soil-borne diseases contribute to succession in foredune vegetation. *Nature* 362: 53-56.
- Van der Putten, W. H., Bardgett, R. D., Bever, J. D., Bezemer, T. M., Casper, B. B., Fukami, T., Kardol, P., Klironomos, J. N., Kulmatiski, A., Schweitzer, J. A., Suding, K. N., van de Vooorde, T. F. J. & Wardle, D. A. (2013). Plant-soil feedbacks: the past, the present and the future challenges. *Journal of Ecology* 101: 265-276.

- Varma, A. (2008). *Mycorrhiza*. Springer, Heidelberg. 797 pp.
- Vierheilig, H., Coughlan, A. P., Wyss, U. & Piché, Y. (1998). Ink and Vinegar, a Simple Staining Technique for Arbuscular-Mycorrhizal Fungi. *Applied and Environmental Microbiology* 64(12): 5004-5007.
- Walker, L. R. & Del Moral, R. (2003). *Primary Succession and Ecosystem Rehabilitation*. Cambridge University Press, New York. 442 pp.

11. SUPPLEMENTARY MATERIAL



Appendix 1. Aerial photographs showing the study area and some of the sampled islands in detail. **A)** Marshy islands of Bogense. **B)** Sandy islands of Bogense. **C)** Odense. **D)** Bogense 14. **E)** Bogense 7 (left). **F)** Odense 16. Orthoimages were provided by Danmarks Miljøportal.

Plot ID	Coordinates	Age	Type	% OM	% WC	Conductivity (μS)	NO ₃ (mg/g)	PO ₄ (mg/g)	NH ₄ (mg/g)
Bogense N	575.773,73 X 6.161.353,86 Y	100	Marshy mainland	26,037	72,07	4006	0,276843	0,127986	0,025568
Bogense 14	581.287,74 X 6.164.115,57 Y	41	Marshy island	10,438	49,86	2930	0,024	0,304221	0,046632
Bogense 15	581.553,08 X 6.164.132,82 Y	19	Marshy island	19,584	-	5090	0,062	0,128235	0,307655
Bogense S	569.879,26 X 6.159.226,45 Y	100	Sandy mainland	1,112	3,64	44	0,162258	0,146537	0,196507
Bogense 3	569.381,75 X 6.160.275,45 Y	54	Sandy island	1,256	2,36	380	1,917	1,516928	0,258632
Bogense 7	572.874,61 X 6.162.721,99 Y	12	Sandy island	2,202	5,78	652	3,717	1,446	0,571
Odense E	602.341,04 X 6.152.080,42 Y	100	Sandy mainland	0,560	1,22	73	1,025522	0,586371	0,513283
Odense 16	602.384,89 X 6.150.165,89 Y	19	Sandy island	0,479	0,75	107	0,499191	0,313415	0,019416
Odense 18	601.349,32 X 6.152.490,07 Y	23	Sandy island	0,424	0,33	50	0,048902	0,07962	0,019839

Appendix 2. Table representing the abiotic data of each study site. % OM shows the data of organic material. % WC shows the soil water content.

Data were provided by Jespersen et al., 2018.

Species	Origin	Wild relative	Symbiotic strategy	% WC	Survival %
<i>Lolium perenne</i>	Commercial seeds	<i>Festuca rubra</i>	Mycorrhizal	85,87	100
<i>Sorghum vulgare</i>	Commercial seeds	<i>Puccinellia maritima</i>	Mycorrhizal	88,34	100
<i>Trifolium alexandrinum</i>	Commercial seeds	<i>Trifolium repens</i>	Mycorrhizal + <i>Rhizobium</i>	89,49	91,7
<i>Lotus corniculatus</i>	Commercial seeds	<i>Trifolium repens</i>	Mycorrhizal + <i>Rhizobium</i>	79,57	95,8
<i>Sinapis alba</i>	Commercial seeds	<i>Lepidium ruderale</i>	Non-mycorrhizal	81,36	95,8
<i>Spinacia oleracea</i>	Commercial seeds	<i>Beta vulgaris</i>	Non-mycorrhizal	77,70	50
<i>Sedum acre</i>	Bogense 3	-	Non-mycorrhizal	80,79	95,8
<i>Sedum acre</i>	Bogense 7	-	Non-mycorrhizal	86,22	100
<i>Honckenya peploides</i>	Bogense 3	-	Non-mycorrhizal	83,90	33,3
<i>Honckenya peploides</i>	Bogense 7	-	Non-mycorrhizal	90,79	75

Appendix 3. Table representing the characteristics of each studied plant species. % WC shows the water content of the plants.